IMPACTS OF HOST BIOLOGY ON THE URBAN ECO-EPIDEMIOLOGY OF WEST NILE VIRUS

BY

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DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Ecology, Evolution, and Conservation Biology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2014

Urbana, Illinois

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ABSTRACT

Disease transmission is inherently ecological, requiring ecological interactions among vectors, hosts and pathogens. Disease ecology as a field, however, has only emerged within the past several decades. Despite significant advances in the study of ecology of infectious diseases in the past twenty years, many questions remain about how host and vector ecology shape patterns of disease outbreaks. For my dissertation, I used radio telemetry, mosquito trapping, and field experiments to study how aspects of host and vector ecology impact disease transmission in a model vector-borne disease system, West Nile virus, in the greater Chicago area. First, I found that social behavior of an important host species, the American robin (*Turdus migratorius*, hereafter robins), and results in decreased individual risk of West Nile virus exposure for social individuals compared to non-social birds. Using sentinel birds, I determined that birds which did not participate in communal roosts had higher risk of exposure to West Nile virus than birds within communal roosts. Decreased exposure to West Nile virus was mediated by a 40 fold decrease in encounters with infected mosquitoes for birds inside communal roosts, despite no significant differences between vector infection rates or abundances inside and outside of communal roosts. Second, I found significant correlations between habitats used by robins early in the summer and habitats associated with elevated vector West Nile virus infection rates several weeks later. Previous work has found evidence supporting the importance of robins to the transmission of West Nile virus, but also found that vector infection rates peak during periods when vectors are not actively feeding on robins. My findings support the importance of robins to the transmission cycle of West Nile virus, and also indicate interactions between vectors and hosts vary throughout the transmission cycle of West Nile virus. Furthermore, my results indicate vector-host interactions resulting in transmission of West Nile virus occur early in the

season before outbreaks of West Nile virus are observed in wildlife or humans. Third, I observed no relationship between exposure to internal parasites and immune function in nestling robins. Infection by helminth parasites can over activate the T-helper cell 2 (Th2) immune response, leaving hosts more susceptible to infection by viruses or bacteria. Since juvenile robins are implicated in the West Nile virus transmission cycle due to lack of previous exposure to the virus, I hypothesized the high prevalence of internal parasites may suppress immune function in young birds resulting in elevated susceptibility to other infections. I found no evidence for this mechanism acting in nestling robins, suggesting the role of juvenile robins in the transmission cycle of West Nile virus may be mediated more by behavioral differences between juveniles and adults such as habitat selection, defensive anti-mosquito behaviors, or utilization of communal roosts than by physiological differences in immune function.

In summary, I found evidence for decreased West Nile virus transmission associated with social behavior in robins, and support for vector-host interactions early in the transmission cycle of West Nile virus resulting in elevated vector infection rates late in the season. These results suggest a selective advantage to social behavior in hosts infected by vector-borne diseases, which runs contrary to historical theory on the selective pressure of disease on social behavior. Furthermore, my findings highlight the importance of fine-scale studies of the ecology of vectors and hosts for understanding spatio-temporal heterogeneity in disease transmission, as significant time-lags may occur between host or vector exposure and detectable infection.

ACKNOWLEDGMENTS

My Ph.D. would not have been possible without the support of many great scientists, colleagues, and my friends and family. I would first like to thank my advisor, Jeff Brawn. From our first phone interview when he asked me if I had hands, I knew we would work well together. Jeff supported me financially during long field seasons, kept me laughing when the going got tough, and gave me enough guidance to keep me on track laced with enough freedom to develop my own projects. I will always be grateful to Jeff for his willingness to take me on as a Ph.D. student when I was fresh out of college with no serious field experience. I will also never forget the sound of him screaming about his imminent death while zip-lining in South Africa, another opportunity for which I am eternally grateful. I hope Jeff and I will remain colleagues and friends for years to come. I would like to thank my committee members Brian Allan, Marilyn O'Hara, Mike Ward and Pat Weatherhead for their logistical support and guidance. I am also grateful for the support and feedback from the Bird Lab group, faculty and students. The diversity of projects and talent present in those Friday lunch meetings is truly astounding, and I am lucky to have been a part of it.

I would also like to thank my collaborators on the West Nile virus project, Tony Goldberg, Tavis Anderson, Gabe Hamer, Ned Walker, Uriel Kitron, and Marilyn O'Hara. My project would not have been possible without the groundwork laid by the early years of the study, or without the lab support from the Walker and Hamer labs. I am also grateful to the small army of field assistants, vet students and other graduate students who spent countless hours sorting through mosquitoes and tracking birds with me: Carl Hutter, Lauren Kmieciak, Shawn Janairo, Amanda Dolinski, Patrick Kelly, Dana Johnson, Jessica Girard, Alicia Hines, Charles Hartley, Nichar Gregory, Tina Whitney and many other volunteers. I would particularly like to

thank Diane Gohde and Tim Thompson for their years of hard work on the project. I am also grateful for the friends I have made while working on the West Nile virus project, Christina Newman, Marija Gorinshteyn and Allie Gardner. While our field work was hardly dull, it would not have been the same without the jokes and laughter borne of sleep deprivation and too many mosquito bites. I look forward to seeing how their careers progress and hope we will stay in touch in coming years.

Finally, I am eternally grateful to my friends and family for supporting me in this endeavor. I am grateful for the support of my uncle, Charles Krebs, who gave me my first chance to do biological field work, gave a guest lecture at University of Illinois, and provided feedback on my thesis. I'd also like to thank my oldest sister Laura for being continually there for me. I am grateful for my parents, Steve and Susie and my other sister Angel, who have supported me in this endeavor. I am thankful to my friends and lab mates Nicole Davros, John Andrews, and Janice Kelly for keeping me sane throughout this process. Despite Nicole's employment many states away, she remains a valuable resource for me personally and professionally. I expect we will spend many future holidays in Minnesota reminiscing about our days at the University of Illinois, although she shouldn't hold out hope for making me vacation in northern Minnesota. John Andrews' zest for life is contagious, and his ability to befriend anyone continues to amaze me. Without him, I would definitely not have had as much fun or met as many people in Illinois as I did, and I look forward to getting a behind the scenes tour at Lincoln Park Zoo from him. Although Janice and I only met my last year at University of Illinois, I have no doubt we will stay close in coming years. I am so glad we met, and without the hours of talking, drinking and playing Final Fantasy with her cockatiels, I'm not sure I would have survived the writing process. I look forward to seeing where her research takes her and

many hours of gaming together in the future.

Funding for my research was provided by the National Science Foundation's Ecology of Emerging Infectious Diseases grant. Further funding was provided by the Illinois Ornithological Society, Wilson Ornithological Society, and School of Integrated Biology at the University of Illinois.

Last but not least, I would like to thank John Kelm, who has supported me in every possible way one person can support another these last five years. I am amazed by his limitless curiosity and appreciation for the world, and have learned so much from him. He braved field work and mosquitoes, tolerated my crazy field schedule for four summers, put in countless hours traveling back and forth between California and Illinois, and has never once complained. Without him, my time at the University of Illinois would have been far less joy-filled. I am forever thankful for all the support, love and laughter he has given me.

PREFACE

Chapter 1 is written in first-person singular form. Chapter 2 is written as a stand-alone manuscript for publication, and I have retained the plural form for this dissertation. Chapters 2 and 3 are written in the first-person singular form. Chapter 2 is under review in Proceedings of the Royal Society B, and was co-authored with my advisor, Jeff Brawn, and our collaborators on the West Nile virus project: Tony Goldberg, University of Wisconsin-Madison; Gabe Hamer, Texas A&M University; Marilyn O'Hara, University of Illinois at Urbana-Champaign; Tavis Anderson, Georgia Southern University; Uriel Kitron, Emory University; Ned Walker, Michigan State University; Christina Newman, University of Wisconsin Madison. All collaborators provided comments on the chapter. Christina Newman and Tavis Anderson assisted with data collection in the field, and Gabe Hamer ran laboratory analyses.

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CHAPTER 1: INTRODUCTION

Disease transmission in natural systems depends on ecological interactions among hosts and pathogens (Anderson and May 1979; May and Anderson 1979). For diseases that are transmitted among hosts by arthropod vectors, or vector-borne diseases, transmission depends on ecological interactions among hosts, pathogens and vectors (May and Anderson 1979). Beyond ecological interactions necessary for the spread of diseases, host mortality from disease can significantly alter the structure of ecological communities (Dobson and Hudson 1986; Kilpatrick et al. 2007). Thus, the study of disease and disease transmission is intrinsically ecological (Anderson and May 1979; Dobson and Hudson 1986; Garnet and Holmes 1996; Raffel et al. 2008).

Despite the ecological interactions inherent in the study of disease, disease ecology as a separate field of study has only emerged within the past thirty years (Anderson and May 1979; Dobson and Hudson 1986; Raffel et al. 2008) and development of the field has coincided with increased emergence of novel infectious diseases in humans and animals (Daszak, Cunningham and Hyatt 2000; Jones et al. 2008; Wilcox and Gubler 2005). Increasing evidence supports anthropogenic changes to the environment as one of the main causes of disease emergence due to changes in ecological interactions between hosts and pathogens (Kilpatrick and Altizer 2010; Taal et al. 2012; Kilpatrick 2011; Crowl et al. 2008; Jones et al. 2008).

Disease emergences can occur when ecological interactions between hosts, pathogens, or vectors are altered (Kilpatrick 2011). Movements of infected individuals have been implicated in the movement of HIV out of Africa (Zhu et al. 1998). Habitat fragmentation can alter interactions between species resulting in novel host-pathogen interactions, such as the habitat changes leading to the emergence of Lyme disease in North America (Taal et al. 2012).

Introductions of new disease vectors can cause outbreaks of diseases carried by that vector, which was observed with the introduction of mosquito vectors of avian malaria to Hawaii (Atkinson et al. 1995). Climate change can also shift the geographic ranges of vectors and hosts such that they interact with novel pathogens or other potential host species (Wilcox and Gubler 2005). Thus, understanding the ecological interactions among hosts, pathogens, or vectors resulting in disease transmission is a central to the study of disease ecology.

Diseases of wildlife are a major source of emergent infectious diseases, and historically infectious diseases of animals have been the largest source of emergent pathogens of humans and livestock (Gratz 1999; Jones et al. 2008; Taylor, Latham and Woolhouse 2000; Wolfe et al. 2007). Wildlife can serve as 'reservoir hosts', or hosts capable of spreading pathogens without readily displaying signs of illness (Taylor 1984; Corbel 1997). Novel ecological interactions between humans or livestock and infected wildlife reservoirs can result in outbreaks of previously unidentified diseases because wildlife hosts which have co-evolved with a disease are often less negatively impacted by infections than hosts which have not co-evolved with a pathogen (Taylor 1984; Corbel 1997). Disease ecologists seek to identify reservoir hosts of infections, understand the transmission cycle, and identify underlying ecological conditions which facilitate or drive the transmission cycle of a disease.

One key factor in understanding the transmission cycle of a disease is the mode of disease transmission. Early models of disease transmission considered differences between pathogens which are transmitted from host-to-host (direct transmission), diseases which are transmitted through the environment (indirect transmission), or diseases that are transmitted between hosts by arthropod vectors (vector-borne transmission, Anderson and May 1979; May and Anderson 1979). As understanding of how diseases behave in natural systems has increased,

more complex models have been developed that include demographic parameters of vectors as well as hosts, or disease systems with multiple hosts (Kilpatrick and Altizer 2010). Theoretical models of disease transmission allow disease ecologists to make predictions about how diseases spread under changing ecological conditions based on their knowledge of how a disease is transmitted between hosts.

Transmission of vector-borne diseases is more ecologically complex than directly transmitted infections because of the interactions between hosts, vectors and pathogens necessary for a disease to spread (Kilpatrick 2011; Eisen and Eisen 2007; Hamer et al. 2009; Hamer et al. 2011). Moreover, because arthropod vectors of diseases are particularly sensitive to environmental conditions, outbreaks of vector-borne diseases can exhibit stronger seasonality and be prone to more varied spatio-temporal distributions than directly transmitted diseases (Eisen and Eisen 2007). Understanding the ecology of vectors and hosts in vector-borne disease systems is necessary to understanding the patterns of disease transmission observed during vector-borne disease outbreaks. For my dissertation, I studied ecological interactions between vectors and hosts of an emerging infectious disease, West Nile virus (WNV), to examine how behavioral ecology of hosts, spatial ecology of hosts and vectors, and host physiology influence the spread of a vector-borne disease in wildlife. Although WNV infects many wild bird species (Komar et al. 2003), my research focused on American robins (*Turdus migratorius*) due to their status as an important reservoir host for West Nile virus in many parts of North America (Kent 2009; Kilpatrick et al. 2006; Hamer et al 2008).

WNV is a vector-borne pathogen that is maintained in an enzootic cycle between *Culex* species mosquitoes and wild birds (Komar et al. 2003; Hamer et al. 2011). Studies of the transmission dynamics of WNV in the Chicago region, where my work took place, and

elsewhere have shown that *Culex* mosquitoes feed preferentially on American robins over other available avian host species (Hamer et al. 2008; Hamer et al. 2009; Simpson et al. 2009; Kilpatrick et al. 2010). Robins are highly competent hosts for WNV (Komar et al. 2003), which contributes to the disproportional impact of robins to the WNV transmission cycle in the greater Chicago suburbs (Hamer et al. 2011). WNV has become endemic across North America since its introduction in 1999 (Kilpatrick 2011), and is a useful and widely applicable model system to study the transmission dynamics of zoonotic vector-borne illnesses (Simpson et al. 2012).

The 'encounter-dilution effect' posits that host aggregations dilute insect bites across all individuals within a group (Turner and Pitcher 1986; Mooring and Hart 1992), however evidence is mixed regarding whether the dilution of insect bites across group members results in decreased exposure to vector-borne diseases for social individuals (Brown et al. 2001; Ward et al. 2006; Loehle 1995; Ratti et al. 2006; Torr et al. 2007). In chapter 2, "Decreased Risk of Exposure to a Vector-Borne Pathogen in American Robin (*Turdus migratorius)* Communal Roosts," I present the results of an experimental study of the role of host social behavior on the transmission cycle of a vector-borne disease. Robins are known to form large communal roosts throughout the summer, when WNV transmission peaks (Hamer et al. 2008; Howell 1942; Eiserer 1984). *Culex* vectors of WNV exhibit peak host seeking behaviors during the period when robins are in communal roosts (Savage et al. 2008), suggesting communal robin roosts may be where WNV transmission occurs. I hypothesized that birds participating in communal roosts would dilute infectious vector bites across all roost members, thereby decreasing individual risk of exposure to WNV within communal roosts compared to outside of communal roosts. I also trapped mosquitoes inside and outside of roosts to measure vector infection rates and abundance at roost

and non-roost sites, and estimated the *per capita* encounter rate of hosts with infected vectors at roost and non-roost sites.

Outbreaks and transmission patterns of West Nile virus (WNV) in the suburbs of Chicago, has been extensively studied (Hamer et al. 2008; Hamer et al. 2009; Hamer et al. 2011; Girard et al. 2011; Amore et al. 2010; Ruiz et al. 2010). Nonetheless, the timing and location of vector-host interactions driving the seasonal transmission and amplification cycle of WNV in this system remains unclear. In chapter 3, "Early Season Vectors-Host Interactions Drive Late Season Patterns of West Nile virus Distribution", I present the results of a study examining seasonal habitat selection by robins and *Culex* to determine how vectors and hosts use habitat in the greater Chicago area throughout the transmission season of WNV. I examined patterns of habitat selection by vectors and hosts to determine whether similar habitat requirements lead to vector-host interactions, and also looked for correlations between habitat characteristics associated with elevated vector infection rates and areas used by birds at time-lagged intervals. I hypothesized that patterns of habitat selection between vectors and host would be different, and that habitat use by robins early in the season would be significantly correlated to WNV infection rates in vectors later in the season. I also looked for evidence of habitats associated with areas of elevated vector infection rates.

The transmission of West Nile virus in parts of North America may be disproportionately impacted by immune function in juvenile American robins due to a lack of protective immunity to WNV upon fledging (Loss et al. 2009; Kilpatrick et al. 2010), high competence for WNV (Komar et al. 2003), and potentially under-developed immune systems upon fledging due to energetic trade-offs between immune development and growth observed in other passerines (Brommer 2004; Soler et al. 2003; Pitala et al. 2010; Brommer et al. 2011). Early exposure to

ecto-parasitism stimulates investment in immune function in nestling birds (Moller and Eritzoe 2002; Pitala et al. 2010; Christe, et al. 1998). Whether early exposure to internal parasites potentiates or suppresses host immune function is less clear, because some parasites secrete chemicals capable of subduing the host's immune response to facilitate tissue invasion by the parasite (Barriga 1984; Degen et al. 2004). Hyper-activation of the immune system to parasites can lead to suppression of other immune pathways, causing a host to become more susceptible to viral or bacterial infections (Degen et al. 2004; Ezenwa et al. 2010). In chapter 4, "Parasitism and Immune Function in Nestling American Robins (*Turdus migratorius*)", I present the results of a medication study examining the interactions between parasitism, investment in immune function, and growth. I hypothesized that early exposure to internal parasites in nestling American robins may over stimulate investment in the T-helper cell 2 (Th2) immune pathway associated with response to parasitism, which is associated with increased susceptibility to pathogens mediate through the Th1 immune pathway, such as viruses or bacterial infections. I also expected to see evidence of release from Th1 pathway suppression with a reduction in parasite burden.

I found evidence for the action of the encounter-dilution effect in vector-borne disease systems, as I found evidence for decreased transmission of WNV within communal robin roosts. My results also indicate that early season transmission of WNV between *Culex* vectors and robins likely drives the seasonal amplification seen in mid-to-late summer in the Chicago WNV system. I found no evidence for interactions between internal parasitism and immune function in nestling robins. This research resulted in the first experimental evidence for the encounter-dilution effect acting in vector-borne disease systems, suggesting that contrary to historical

theory disease can act as a selective force for social behavior in an organism but the selective pressure of disease on social behavior may depend on the mode of disease transmission.

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CHAPTER 2: DECREASED RISK OF EXPOSURE TO A VECTOR-BORNE PATHOGEN IN AMERICAN ROBIN (TURDUS MIGRATORIUS) COMMUNAL ROOSTS

ABSTRACT

Animals can decrease their individual risk of predation by forming social groups. The encounter-dilution hypothesis extends the potential benefits of social behavior to biting insects and vector-borne disease by predicting that the *per capita* number of insect bites should decrease within larger host groups. Although vector-borne diseases are common and can exert strong selective pressures on hosts, there have been few tests of the encounter-dilution effect in natural systems. We conducted an experimental test of the encounter-dilution hypothesis using the American robin (*Turdus migratorius*), a common host species for the West Nile virus (WNV), a mosquito-borne pathogen. By using sentinel hosts (House Sparrows, *Passer domesticus*) caged in naturally occurring communal roosts in the suburbs of Chicago, we assessed the risk of WNV seroconversion inside and outside of roosts. We also estimated per capita host exposure to infected vectors inside roosts and outside of roosts. Sentinel birds caged inside roosts seroconverted to WNV more slowly than those outside of roosts, suggesting that social groups decrease *per capita* exposure to infected mosquitoes. These results therefore support the encounter-dilution hypothesis in a vector-borne disease system. Our results suggest that disease-related selective pressures on social behavior may depend on the mode of disease transmission.

INTRODUCTION

A "selfish herd" may attract more predators owing to greater visibility of the group, but individuals "dilute" their risk of predation across all other group members [1]. This hypothesis has been extended to biting insects and risk of exposure to vector-borne disease in a concept

known as the "encounter-dilution effect" [2,3]. By distributing the risk of insect bites across many individuals, social groups decrease the *per capita* vector biting rate [4,5]. Given that a host's risk of disease exposure increases with the number of insect bites received [6,7], social groups may also decrease an individual's risk of exposure to vector-borne pathogens.

Empirical evidence for the encounter-dilution effect is mixed and largely observational, although models predict the encounter-dilution effect in vector-borne disease systems [8,9]. Comparative studies of social species and nonsocial congeners have found higher prevalence of malaria and arboviruses in social species of bird and primates [10-13], contrary to the predictions of this hypothesis. Conversely, studies of livestock herds and avian flocks indicate that social behavior does decrease *per capita* vector biting rates [5,14,15]. Nonetheless, definitive evidence of decreased host exposure to vector-borne pathogens as a result of social behavior is scant [but see 7]. Because vector-borne diseases can exert significant fitness effects on hosts [16,17], these effects could provide selection favoring social behavior but this hypothesis currently lacks empirical support.

West Nile virus (WNV) is a vector-borne pathogen transmitted by *Culex* spp. mosquitoes [18]. WNV is maintained in a seasonal transmission cycle between vectors and wild birds [19,20]. The American robin (*Turdus migratorius*, hereafter 'robin') is an important host species in the WNV transmission cycle due to high competency, i.e., the ability of robins to transmit and contract the disease during interactions with vectors [21,22]. Robins form communal roosts throughout their breeding season [23,24], which coincides with the peak transmission season of WNV. Previous work suggests communal robin roosts may enable localized transmission of WNV between birds and vectors [25].

We examined communal robin roosts in the west Chicago suburbs, a WNV "hotspot," [22,26] and conducted a field experiment to test the encounter-dilution effect . By housing sentinel birds in cages inside and outside of communal roost sites and trapping mosquitoes at roost and non-roost sites, we were able to test the following predictions: if the encounter-dilution effect acts on vector-borne disease transmission, then sentinel birds in communal roosts should have lower risk of exposure to WNV than those away from communal roosts. We also predicted a lower *per capita* vector index, (i.e., the estimated number of interactions a host has with infected mosquitoes per night) for birds in communal roosts.

METHODS

We located five large (200-20,000 birds) communal robin roosts in Cook County, Illinois between May and October of 2010-2012. We trapped mosquitoes inside communal roosts and in residential areas, urban parks, and natural areas away from roosts. Vector infection rates from mosquitoes captured in 2012 were also used to estimate the *per capita* vector index [27], i.e., the number of infected mosquitoes encountered per host per night at roost and non-roost sites.

Estimating Risk of West Nile Virus Exposure

To estimate host risk of exposure to WNV at each site type, we conducted an experiment using sentinel birds. Sentinel birds (typically chickens or other galliformes) are commonly used by public health agencies to survey for transmission of vector-borne diseases such as WNV [28-30]. For this study, we used house sparrows (*Passer domesticus*, hereafter sparrows) as the sentinel species. We used sparrows rather than American robins because sparrows are competent hosts for WNV [20], contribute significantly to the WNV transmission cycle in Chicago as determined from vector blood meal analysis [19], can be held in captivity with reasonable effort, and unlike robins, are not protected by statute in North America.

In 2012, we selected three roost sites and three non-roost sites. We captured, individually marked with colored leg bands, and drew blood samples from free-living sparrows to screen for previous WNV exposure. After initial blood draws, we housed sparrows in flight cages at a field laboratory until screening for previous exposure was completed. We screened blood samples for WNV RNA using a quantitative RT-PCR and WNV antibodies using an inhibition ELISA as previously described [26], with one amendment being that viral RNA was extracting using the MagMAX Viral RNA Isolation Kit (Applied Biosystems, Foster City, California).

Birds that tested negative for WNV antibodies were transferred in groups of five to each of six field cages, three in roost sites and three in non-roost sites. Field cages were built from commercially available bird cages elevated onto four 3 meter galvanized steel pipes. We coated the anchor poles with machine grease to prevent disturbance from the public or mammalian predators.

We deployed sentinel cages by the third week of July, 2012, after which we drew blood samples by jugular venipuncture from each bird weekly for the next eight weeks. The timing of this experiment coincided with the historical period of peak WNV transmission in our study area [26]. Samples drawn from birds in field cages were tested as described above on a weekly basis. We maintained a group of WNV unexposed sparrows protected from exposure to vectors as 'reserve' birds. As birds housed in field cages became exposed to WNV, they were removed and replaced with unexposed birds from the reserve group. All birds were provided food and water *ad libitum.* Animal care, use, and housing were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois at Urbana-Champaign, protocol #12047.

Mosquito Sampling

We captured mosquitoes inside and outside communal roosts from 2010 to 2012 and

compared relative abundances of *Culex* spp. (hereafter *Culex*) and WNV mosquito infection rates inside and outside of roosts. In 2012, we estimated the *per capita* vector index, the number of infected vectors encountered per host per night inside and outside communal roosts. CDC carbon dioxide (CO_2) -baited light traps were used to estimate abundances of host-seeking female mosquitoes of various species, as the CO_2 given off by the traps simulates respiration by hosts. Infusion-baited gravid traps were used to enhance capture of *Culex* vectors already infected with WNV [31]. To estimate per capita biting rates of *Culex* on hosts, we utilized bird-baited traps. Bird-baited traps are modified CDC light traps that use a single bird as bait in place of CO_2 and attract mosquitoes such as *Culex* that prefer feeding on avian hosts [32].

We deployed CDC CO_2-baited light traps and infusion-baited gravid traps for one night per week at each sampling site from the beginning of June through mid-October in 2010-2012. We placed 66 traps inside roosts and 270 traps in non-roost areas: this equated to 140 traps in 2010, 133 in 2011, and 63 in 2012.We identified all captured mosquitoes and pooled them by species, site of collection, and blood-fed status. We then tested pools for WNV infection following the protocols previously described [26].

We constructed bird-baited mosquito traps following Emord and Morris [32]. A single sparrow was placed in the cage attached to the trap, and the trap was placed at least 25 meters away from one of the sentinel bird cages for one night per week during the course of the sentinel bird study, which equated to six bird-baited trap nights per week over eight weeks. To protect bait birds from mosquitoes, all cages were lined with insect screening. Each bait-bird was used in a trap only one night per week, and bait birds were kept separate from 'reserve' birds used in the sentinel bird experiment.

Analyzing the Risk of West Nile Virus Exposure

Relative risk of sentinel bird exposure to WNV inside and outside communal roosts was analyzed using a Cox-proportional hazards mixed model in the coxme package in R. The proportional hazards model assumes the risk of an event occurring, in this case WNV infection of a given bird, is the same across all time intervals. We tested this assumption using the survival package in R, and as this assumption was not violated ($p > 0.1$) we used the proportional hazards model. The length of time an individual remained unexposed to WNV was analyzed as dependent on a site type (roost or non-roost) with a random effect for individual birds nested within cage [33].

Estimating Vector Abundance, Infection Rates and Vector Index

To describe overall *Culex* abundance and infection rates from 2010-2012, we pooled light and gravid traps into 19 spatially clustered groups; 14 outside of roosts and one inside each of five communal roosts. Vector abundance from light traps was estimated as the number of *Culex* captured per light trap night within each spatial group. We derived maximum likelihood estimates of the minimum infection rate (MIR, number of infected mosquitoes per 1,000 mosquitoes trapped) [34] of *Culex* mosquito pools with 95% confidence intervals by spatial group using the Pooled Infection Rate Version 3.0 Add-In [35].

To compare host encounter rates with infected vectors at roost and non-roost sites, we estimate the vector index at each site type in 2012. We calculated the vector index by multiplying the per light trap night estimates of *Culex* abundance by the maximum likelihood estimates of vector infection rates from *Culex* captured in the same light traps during the same time period. To estimate the *per capita* vector index at roost and non-roost sites, we multiplied the MIR estimates from light traps within 200 m of the bird-baited trap by the average number of *Culex* captured per trap night in bird-baited traps in the same time period as sentinel hosts

remained housed in field cages. We used MIR estimates from light traps only to estimate per host vector index because vectors captured in light traps are actively host seeking and therefore capable of transmitting the virus upon their next blood meal. To this end, we assumed host-seeking behavior of *Culex* to be similar when using bird-baited traps and light traps.

To determine whether communal roosts in our study system increased local infection rates in vectors, we compared infection rates inside and outside communal roosts from 2010-2012. We used Poisson regression to analyze *Culex* abundance and MIR in Program R using the lme4 package; the response variable was either *Culex* per trap night or MIR, with fixed effects for site type (roost or non-roost), random effects for week nested in year and spatial group, and an offset term equal to the log of the number of traps set in a spatial group during a given week. The use of the offset term accounts for the variation in trap number set in a given week among spatial groups, allowing analysis of untransformed data [36]. For mosquito abundance estimates from bird baited traps and the per host vector index we used Poisson regression; we included site type as a fixed effect and a random effect for week.

RESULTS

Risk of Host Exposure to West Nile virus and Vector Indices

Between July and September 2012, we placed 23 sentinel birds in cages near communal roosts and 25 in non-roost cages. Only three sentinel birds near roosts seroconverted to WNV, whereas 11 birds in non-roost cages seroconverted. At every sentinel cage at least one sentinel bird seroconverted to WNV during the experiment; therefore *Culex* did feed on sentinel sparrows even at roost cages when numerous robins were present. The risk of WNV exposure for sentinel birds caged within roosts was significantly lower than for birds caged in non-roost locations (z = -2.17, p = 0.03, Figure 1).

Estimated *per capita* vector encounter rates with hosts in 2012 were consistent with results of the sentinel experiment. Vector encounter rates were significantly greater away from roosts (z = -9.568, $p < 0.001$). Moreover, the vector index, i.e., the number of infected *Culex* captured per trap night, was approximately one third of that within communal roosts than outside of communal roosts (z = -17.4, $p < 0.001$, Figure 2A). The average number of *Culex* per bird-baited trap night within communal roosts was 7.56, with a range of 0- 43 mosquitoes. At non-roost sites, the average number of mosquitoes in bird-baited traps was 19.64, ranging from 0- 92 mosquitoes. The average estimated *per capita* vector index was 1.1 infected vectors per host per night outside of roosts, which was over 40 times higher than the vector index inside roosts (0.025 infected vectors per host per night, Figure 2B).

Vector Abundance and Infection Rates at Roost and Non-Roost Sites

Our mosquito sampling effort totaled over 4,000 trap nights of light and gravid traps across three years. We captured 18,158 *Culex* mosquitoes in light traps from 2010-2012, which we used to estimate abundances of host-seeking vectors inside and outside of communal roosts. With added sampling using gravid traps, we estimated Minimum infection rate (MIR) estimates from 40,761 *Culex* mosquitoes. Estimated *Culex* abundances were similar at roost and non-roost sites across all three years (z = 0.16, $p > 0.05$, Figure 3 A-C). The estimated MIR in *Culex* did not differ between roost and non-roost sites (z = -0.22, $p > 0.05$, 3 D-F).

DISCUSSION

Our results indicate that the individual risk of exposure to WNV is lower within communal roosts than outside of them. This finding supports the encounter-dilution hypothesis for vector-borne disease transmission. Our data also suggest that decreased individual risk was mediated by decreased *per capita* interactions with infected vectors within social aggregations.

Furthermore, *Culex* abundance and MIR did not differ between roost and non-roost sites across three years of observation, indicating host aggregation does not enable WNV transmission in our system.

Increased exposure to directly transmitted diseases has been considered a selective force acting against sociality [37], but our results suggest that the mode of disease transmission determines the relationship between disease and host sociality. Disease transmission models often consider how transmission responds to host density, which typically falls into one of two categories: density dependent or frequency dependent transmission. Diseases transmitted directly from host-to-host or through the environment (e.g. soil or water contamination) are typically density dependent, meaning locally greater densities of hosts leads to increased disease transmission [38,39]. Vector-borne disease transmission by contrast is frequency dependent, such that the proportion of infected individuals (vectors or hosts) has more impact on disease transmission than the absolute numbers of hosts or vectors[40,41]. For example, risk of exposure to WNV depends on the ratio of infected vectors to the number of susceptible hosts, because hosts contract WNV only from infected vectors. In our system, increasing the number of hosts in a roost without changing the number of infected vectors decreases the ratio of infected vectors to available hosts, apparently leading to decreased risk of host exposure to WNV. Because we observed similar vector infection rates and abundances inside and outside of communal roosts, we found densities of hosts, but not infected vectors, to be greater within communal roosts. Based on our empirical evidence herein, it appears that selective pressure exerted by diseases on host social behavior is conditional on the mode of disease transmission.

Many factors can favor social behavior in animals, including reduced predation risk or increased foraging efficiency in groups [1,3]. Although decreased individual risk of exposure to

WNV is a selective advantage to social behavior in our system, it is likely not the only advantage to communal roosting. Nuisance feeding by biting insects can alter host behavior [42], reduce fitness by increasing energy expended during behavioral defenses [43,44] and reduce breeding success [45,46]. Consequently, decreased exposure to biting insects within communal roosts likely benefits social individuals even in the absence of infection. Under this scenario, the selective advantages of sociality would increase in the presence of vector-borne infections due to compounding effects of vector feeding and vector-borne infections [16,17]. More generally, the role of disease transmission in shaping social behavior is balanced among other selective pressures facing hosts. When exposure to vector-borne diseases decreases host fitness, then social behavior in hosts would be favored as long as fitness gain from decreased vector-borne disease exposure in social individuals offset fitness losses associated with sociality. Similarly, in a species infected by a directly transmitted disease, decreased social behavior would be favored as long as fitness gained from avoidance of the disease were greater than fitness lost by solitary individuals. Thus, the selective advantage of host sociality in vector-borne disease systems is balanced against other major selective pressures acting on hosts, and does not necessarily act in all organisms impacted by vector-borne diseases.

A potential confounder in our experimental design is vector preference for robins over house sparrows [18]. Conceivably, infectious vectors could have fed on robins in communal roosts and avoided the nearby sentinel sparrows. However, sentinel bird exposure to WNV occurred at all sentinel cages throughout the duration of the experiment; therefore, *Culex* were attracted to and fed on sparrows even in the presence of large numbers of robins. Also, the estimated per capita vector index, or number of infected mosquitoes encountered per host per night inside communal roosts was 0.025, compared to 1.1 away from communal roosts.

Therefore, any host species using a communal roost would experience decreased risk of contact with infectious vectors. Finally, amongst a ranking of 25 bird species found to have been fed upon by *Culex* vectors in our study systems, the rate of transmission of West Nile virus was estimated to be highest from American robins and then house sparrows [19], indicating that *Culex* vectors feed readily on house sparrows.

Implications for vector-borne disease transmission

How host social behavior influences individual risk of disease exposure may vary among vector-borne disease systems, based on factors such as vector or host mobility. For example, increased host densities in fragmented habitats leads to increased prevalence of Lyme disease in black-legged ticks (*Ixodes scapularis*) and rodent hosts [47], and the prevalence of Buggy Creek virus in swallow bugs (*Oeciacus vicarius*) is greater in larger breeding colonies of cliff swallows (*Petrochelidon pyrrhonota*, [48]). Black-legged ticks and swallow bugs are independently mobile over short distances but depend on hosts for long-distance dispersal [49-51]. In both systems, long-distance movement of vectors is limited by movements of hosts themselves (deer for ticks or on adult swallows for swallow bugs). In contrast, *Culex* mosquitoes are capable of independent dispersal of up to 3 km [52]. When vectors are capable of independent dispersal, the potential for a single infected vector to infect multiple hosts in a localized area is decreased, since infected vectors are more likely to disperse before their next blood meal. Thus, the mobility of mosquito vectors of WNV likely contributes to our findings of decreased host exposure to WNV within host aggregations.

The movement of hosts is also an important factor in how vector-borne disease transmission responds to host aggregations. In the above examples, swallow bugs feed largely on nestling swallows that are not capable of dispersing from the breeding colony until they are old

enough to fledge [48]. White-footed mice (*Peromyscus leucopus*) which are key hosts for transmitting *Borrelia burgdorferi* (the causative agent of Lyme disease) to black-legged ticks, also disperse only short distances [53]. In contrast, individual robins vary widely in their use of communal roosts from night to night, such that the composition of a given roost is variable throughout the summer [54]. While a robin may be exposed to WNV within one communal roost, it may not remain at that communal roost to infect additional *Culex* vectors. Given the mobility of both vectors and hosts in the WNV system, communal roosting is unlikely to lead to the type of repeated vector-host interactions that potentiate disease transmission in other systems.

Under the original selfish herd and encounter dilution hypotheses, more central locations within a group are more desirable due to decreased exposure to predators [1] or biting insects [2-4]. Therefore, more favorable central positions should be held by dominant individuals or competed for among group members. Similarly, within communal roosts more dominant individuals should seek to occupy central and more elevated positions, which relegating younger and less dominant birds to less favorable positions [55-57]. Demographic stratification within roosts may have important implications for vector-borne disease exposure, as different vector species feed at different heights within a habitat [58-62]. For example, in one study WNV infection rates in vectors were greater in the canopy than at ground level [62]. While lower positions within a roost may be less favorable in terms of other potential benefits, less dominant individuals relegated to these positions may still benefit from decreased exposure to vector-borne diseases, particularly when vector feeding patterns or infection rates are vertically stratified. We placed sentinel bird cages within communal roosts at central locations based on nocturnal surveys of roost boundaries, suggesting our results are indicative of the benefits received by individual occupying preferred central locations within roosts. We did not assess the impact of

host location within the roost on risk of host exposure to vector-borne diseases. Future work should examine the heterogeneity of host risk of exposure to vector-borne illnesses within groups to examine whether all members of a group benefit equally.

FIGURES AND TABLES

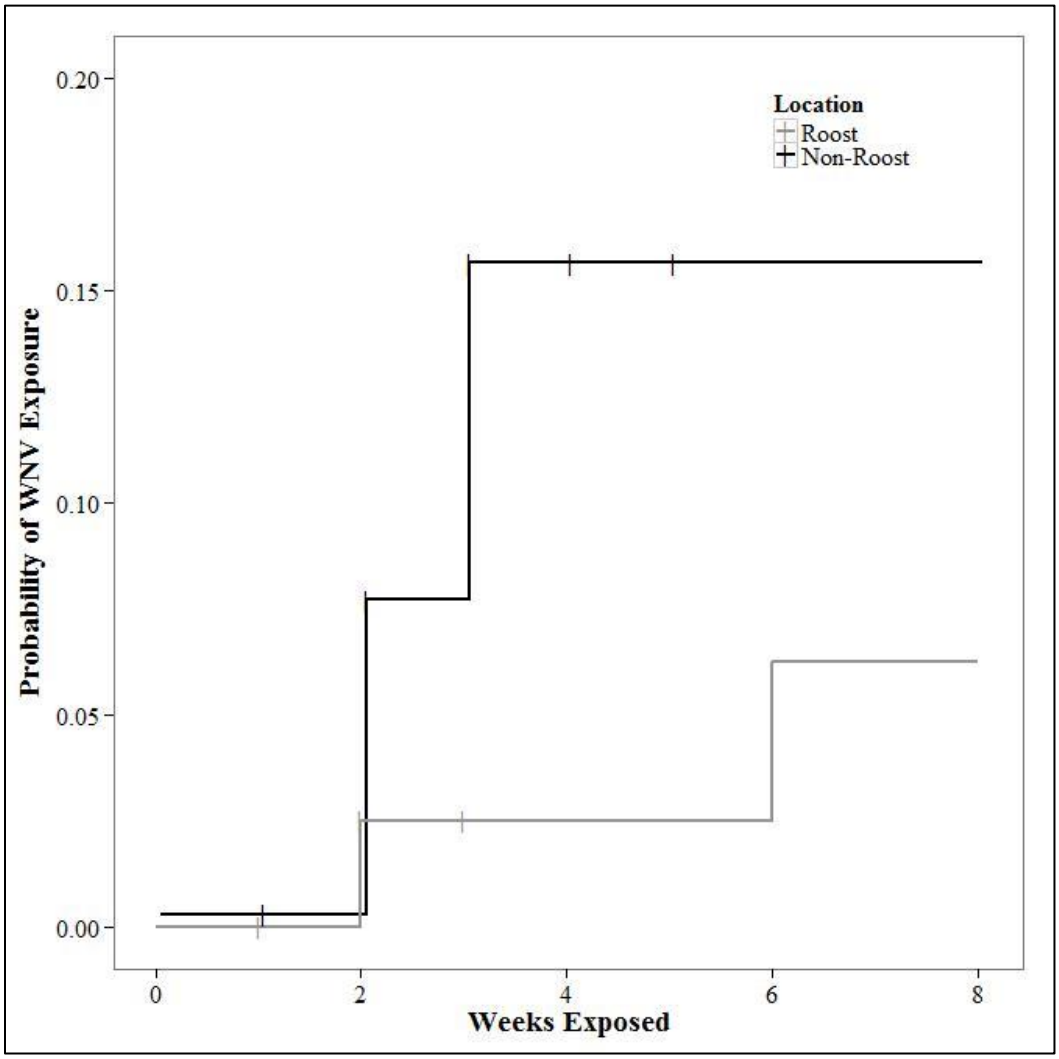

Figure 1. Kaplan-Meier plot of risk to an individual sentinel bird by cage location. Tick marks indicate individuals censored at that interval (e.g. due to non-West Nile related mortality or escape after blood sampling). Plots are offset to ease interpretation.

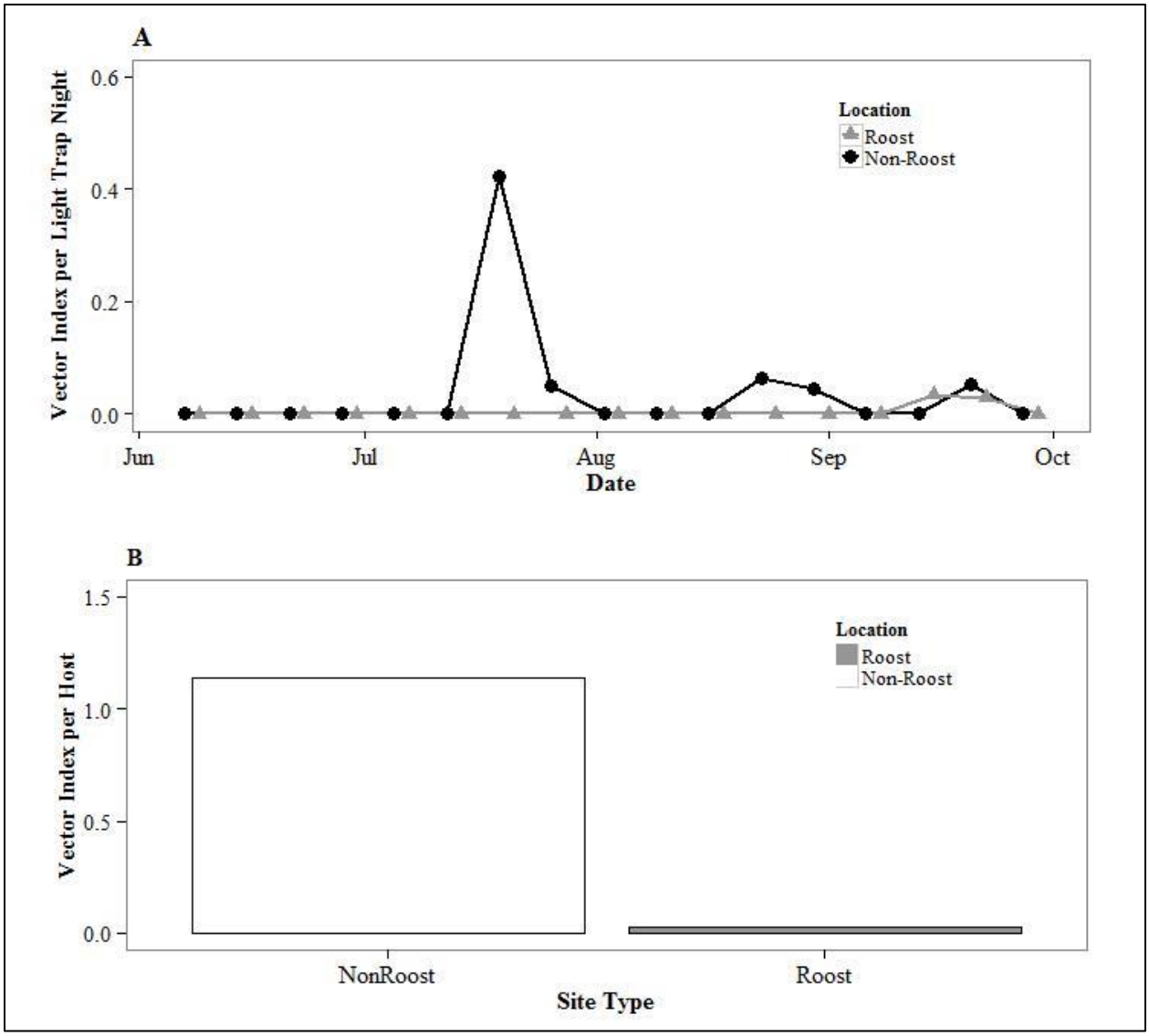

Figure 2. All data were collected in Chicago, Illinois (A) Vector index, or average number of infected *Culex* mosquitoes captured per light trap night, at roost and non-roost locations in 2012 (B) Average *per capita* vector index at roost and non-roost locations, between July and September 2012

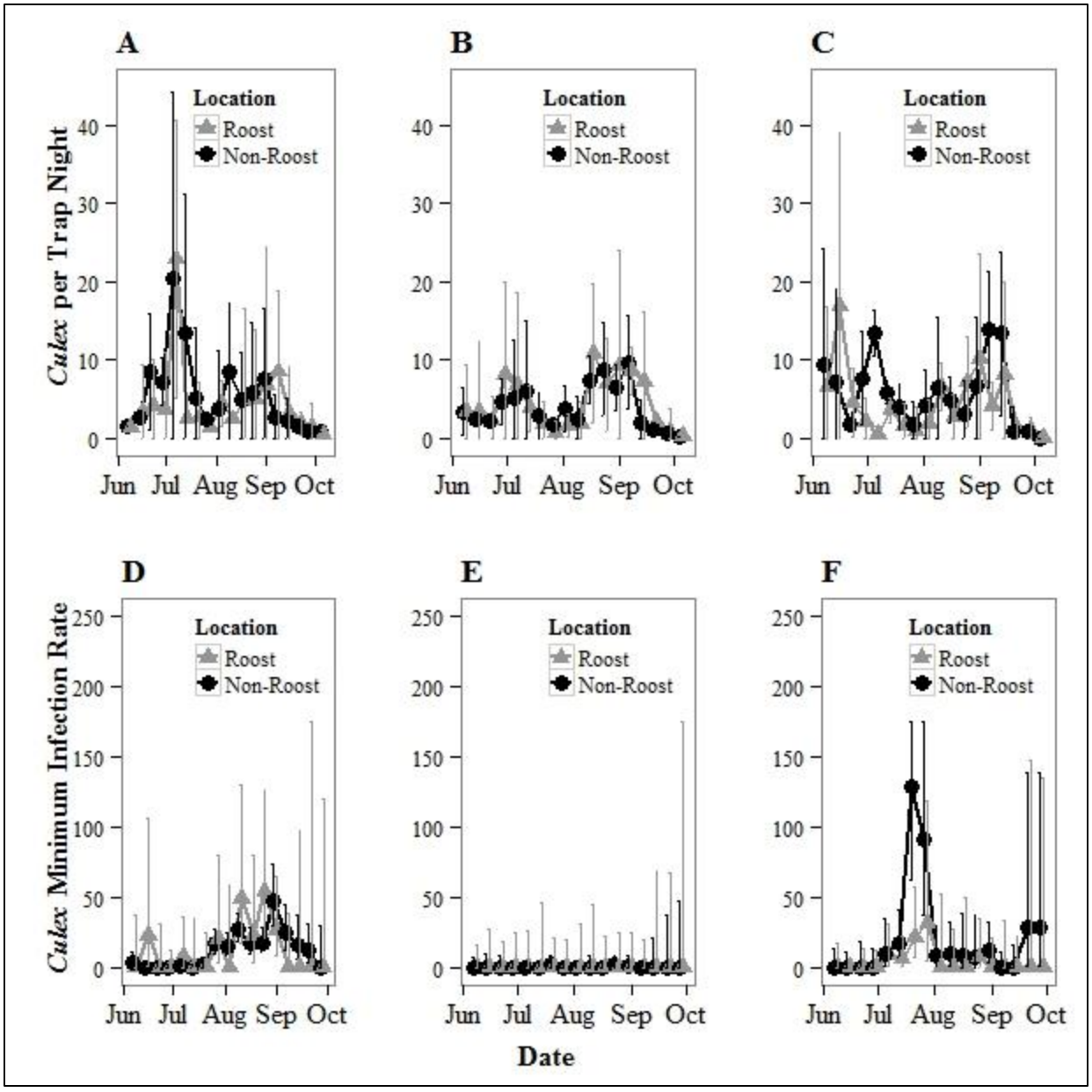

Figure 3. Variation among years in average Culex per light trap night (A) 2010 (B) 2011 and (C) 2012 by trap location, +/- 2 median absolute deviations (D-F) *Culex* minimum infection rate maximum likelihood estimates (D) 2010 (E) 2011 and (F) 2012 by trap location, +/- 95% confidence intervals. All figures based on data collected in Chicago, Illinois

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CHAPTER 3: EARLY SEASON VECTOR-HOST INTERACTIONS UNDERLIE WEST NILE VIRUS DISTRIBUTION LATE IN TRANSMISSION CYCLE

ABSTRACT

Disease transmission requires ecological interactions between hosts and pathogens. In the case of vector-borne diseases, ecological requirements of disease transmission become further complicated by the necessity of interactions between hosts and vectors for disease transmission to occur. Understanding the timing and location of interactions between vectors and hosts involved in vector-borne disease transmission is critical to understanding spatial and temporal patterns of vector-borne disease distribution. Patterns of vector-borne disease prevalence in hosts and vectors are typically highly variable in space and time, making it difficult to determine where and when vector-host interactions occur. In some systems, similar habitat requirements of vectors and hosts drive host aggregation within suitable habitats for vectors, promoting vector-host interactions and resulting in outbreaks of diseases, indicating similar habitat use by vectors and hosts may lead to vector-host interactions resulting in disease transmission. I examined whether similar habitat use by key vector and host species of West Nile virus within the same week could account for spatial patterns of WNV distribution in vectors. I compared habitat use within the same week by American robins (*Turdus migratorius*) and areas with elevated *Culex* spp. mosquito abundance and elevated *Culex* WNV infection rates. I compared habitat use in the same week to time-lagged correlations between habitat characteristics of areas used by robins to areas with elevated *Culex* infection rates. I determine similar overall habitat use by hosts and vectors did not account for variability in *Culex* infection rates, however there were significant time-lagged associations between habitats used by robins and habitats associated with elevated *Culex* infection rates. These results underscore the

importance of robins to the WNV transmission cycle, and further highlight the need for fine-scale spatio-temporal studies of habitat use by vectors and hosts when studying vector-borne disease transmission.

INTRODUCTION

Transmission of vector-borne diseases depends on interactions between infected vectors and competent wildlife hosts. Thus, understanding habitat use by vectors and hosts is crucial to future efforts to predict and control disease outbreaks. Spatial and temporal variability in the distribution of vector-borne diseases is commonly observed (Eisen and Eisen 2007), owing to seasonal variation in abundances of arthropod vectors (Guerra et al. 2002; Reisen et al. 1995), seasonal shifts in the timing or locations of vector-host interactions, or both (Altizer et al. 2006; Simpson et al. 2012; Kilpatrick et al. 2006; Hamer et al. 2011). For example, seasonal associations of avian hosts and vectors of St. Louis encephalitis (SLE) in Florida have been implicated in outbreaks of SLE (Shaman et al. 2002). Thus, understanding habitat selection and use by vectors and hosts throughout the transmission cycle of a vector-borne disease is critical to understanding where and when disease transmission occurs.

Outbreaks and transmission patterns of West Nile virus (WNV) in the suburbs of Chicago, Illinois, have been extensively studied (Amore et al. 2010; Betrolotti et al. 20008; Girard et al. 2011; Hamer et al. 2008; ; Loss et al. 2009; Ruiz et al. 2010). The spatial distribution of WNV in Chicago is characterized by extreme spatial heterogeneity in vector infection rates in developed areas (Ruiz et al. 2004) and yearly peaks of infection in *Culex* mosquitoes, birds and humans that coincide temporally in mid-to-late summer (Hamer et al. 2008). While changes in the frequency of vector host interactions may underlie these patterns, this explanation has not been explored.

Although WNV infects many wild bird species, American robins (hereafter robins) are believed to be keystone host species involved in outbreaks of WNV in the greater Chicago area (Hamer et al. 2011), the northeastern United States, and Colorado (Kilpatrick et al. 2006; Kent et al. 2009). The proportion of *Culex* blood meals derived from robins declines during periods of peak WNV infection in hosts and vectors, despite the importance of robins to the WNV transmission cycle (Hamer et al. 2008; Hamer et al. 2011). Declines in robin derived blood meals by *Culex* vectors late in the summer are consistent across many studies of WNV transmission in the United States (Kent et al. 2009; Kilpatrick et al. 2006). While *Culex* are feeding more on non-robin hosts during periods of peak WNV transmission, close to 90% of blood fed *Culex* infected with WNV had previously fed on robins (Hamer et al. 2011), suggesting blood feeding on robins influences vector infection rates regardless of the timing of the blood meal during the transmission cycle. Since there is an incubation period between when a vector takes an infected blood meal and when the infection becomes detectable in the vector (Amraoui et al. 2012; Richards et al. 2011; Kilpatrick et al. 2008), vector-host interactions may occur prior to elevated infection rates in vectors.

I sampled and compared habitat use of American robins and *Culex* vectors throughout seasonal outbreaks of WNV in the Chicago suburbs from 2010-2012 to test whether temporal coincidence in habitat use between vectors and hosts alone could account for variability in the distribution of WNV. Alternatively, I examined patterns of habitat use by robins and *Culex* for time-lagged associations between habitat use by hosts and infection rates in vectors due to incubation periods between vector exposure and infection (Amraoui et al. 2012; Richards et al. 2011; Kilpatrick et al. 2008).

If temporally coincident habitat use between vectors and hosts leads to vector-host interactions resulting in WNV transmission, then habitat use by hosts and vectors should be similar to each other, and habitats used by vectors and hosts should be similar to habitats associated with elevated vector infection rates. Alternatively, if infection rates in vectors are associated with habitat use by robins earlier in the season, then habitat use by vectors and hosts should differ and habitats associated with elevated vector infection rates should be similar to habitats used by robins earlier in the summer.

METHODS

Study Area

I conducted fieldwork in Cook County, Illinois between May and October of 2010-2012. I trapped mosquitoes in residential areas, urban parks, and natural areas, and at the same sites we conducted mist-netting efforts to capture and place transmitters on robins.

Sampling of mosquito abundance and infection

CDC CO_2-baited light traps and infusion-baited gravid traps were set out one night per week at each sampling site from the beginning of June through mid-October in 2010-2012. Trapping effort consisted of: 140 traps in 2010, 133 in 2011, and 63 in 2012, representing over 4,000 trap nights throughout the duration of the study (see map 1). All captured mosquitoes were identified and pooled according to species, site of collection, and blood-fed status. We tested *Culex* spp. pools for WNV infection following the protocols established in Hamer et al. (2008).

Habitat Use by Birds

Between May and October in 2010-2012, I captured 123 American robins (55 adults and 58 juveniles) and fitted them with harness mounted radio transmitters (SOPB-2028, Wildlife

Materials, Hillsboro IL; Rappole and Tipton 1991). Adult birds and independent juveniles were captured in mist-nets, weighed, measured, and marked with USFWS aluminum bands and color bands. In 2011 and 2012, nestling robins were fitted with transmitters on day 8 post-hatching to monitor habitat use during the post-fledging period, or the period immediately after nestlings leave the nest. The transmitters weighed 1.7 grams, which is approximately 5% of the nestlings' body weight on day 8 post-hatching. Since nestling robins fledge no earlier than day 10 (Howell 1942), by fledging the transmitter is approximately 3% of the fledglings body weight, which is within the 5% body weight limit for transmitters recommended for songbirds (Caccamise and Hedin 1985). Upon release, I sought to locate tagged birds twice daily; once during daylight hours and once after dark to determine roosting locations. I monitored robin locations and movements daily until I was able to confirm battery failure, mortality, or apparent dispersal out of the study area.

Classification of Habitat Types and Habitat Availability

Using orthophotographs of the greater Chicago area (courtesy of The Illinois State Geological Survey, Prairie Research Institute, Champaign, IL), I digitized our study area in ArcGIS 10.1 (Environmental Systems Research Institute, Redlands, CA). I classified habitats as one of fifteen habitat classes: houses, garages and sheds, apartments, schools, other buildings, natural grass, other natural cover, wooded areas, lawns sidewalks and driveways, natural water, retention basins, swimming pools, roads, playgrounds, outdoor tracks.

Habitat selection of host and vectors

Since host-seeking behavior by *Culex* vectors peaks between dusk and dawn (Savage et al. 2008), host-vector interactions are most likely to occur at night. Thus, for each radio-tagged bird, I used all nighttime relocation points to assess roost habitat selection for each week the bird

was tagged. I generated 90% kernel density estimates of the nocturnal home range of each tagged bird for each week, then calculated the proportion of each habitat type within the home range using FragSTATs (McGarigal, Cushman, and Ene 2012).

To examine associations between habitat types and *Culex* abundances or infection rates, I calculated weekly average abundances of *Culex* per trap night for each light trap. For analyses of *Culex* abundance, I excluded data from gravid traps because CDC CO_2 baited light traps attract host-seeking vectors, while infusion baited gravid traps attract *Culex* spp. mosquitoes that have blood fed and are seeking oviposition sites. Therefore, light trap data better estimate abundances of vectors potentially feeding on robins within a certain habitat. I derived maximum likelihood estimates of weekly *Culex* minimum infection rates (MIR, infected mosquitoes per 1,000 individuals trapped, citation, Biggerstaff 2006) at each light and gravid trap. Using the weekly estimates of *Culex* abundance and MIRs at all trap locations, I created kernel density estimates of *Culex* abundance and MIR across our study site for each CDC week from June 1st to October 1st. For areas in the 90th percentile or above for *Culex* abundance and MIR, I repeated processing steps as described for the bird home range estimates.

To assess apparent selectivity in habitat use, I calculated the proportion of each habitat type present in the total extent of digitized land cover in our study area. Using proportions of available and used habitat types for vectors and hosts, I calculated Manly's selection index 95% confidence intervals for each of the land cover types using the adehabitatHS package in R. Manly's selection index (w_i) is the amount of habitat that was used by an animal divided by the amount of habitat available to the animal (Manly et al. 2002); as the index approaches one, the use of a given habitat type becomes equal to its overall availability on the landscape with no apparent selection or avoidance. If a habitat is significantly under-utilized by animals compared

to its availability on the overall landscape, the index will be < 1, and if a habitat is significantly over-utilized, the index will be >1. The term 'habitat selection' will be used for both vectors and hosts for the remainder of the paper, although I acknowledge my findings represent habitat associations and not necessarily cognitive "selection" by vectors or infected vectors comparable to habitat selection by robins.

To compare habitat selection between vectors and hosts, I transformed selection ratios according to the standardization suggested in Manly et al 2002:

$$d_i = \log\left(\frac{o_{ui}}{o_{uj}}\right) - \log\left(\frac{\pi_{ai}}{\pi_{aj}}\right)$$

Where o_{ui} is the proportion of used habitat of type *i*, π_{ai} is the proportion of available habitat *i*, and a single habitat type *j* is used to standardized all other habitat types. After transformation, habitat selection by an individual is represented by a vector of d_i values of length *i-1*, with the value of the *i*th class held constant at 0. All values in this vector are linearly independent, which allows testing for differences in habitat selection between vectors and hosts using MANOVAs.

If habitat selection by vectors and hosts overlaps, then the differences in the d_i vector means are not statistically different from zero and the test returns a non-significant *p*-value. I ran MANOVAs only if both vectors and hosts exhibited evidence for significant habitat selection, as comparing non-significant selection index values would provide no information.

Time-lagged Associations of Host Habitat Use and Vector Infection Rates

To address the potential for time-lagged associations between habitats hosts use early in the season and habitats with elevated vector infection rates later in the season, I examined the correlation between habitat compositions of habitats used by hosts early in the season to habitats

associated with elevated *Culex* MIR and abundance at varying time lags. I ran a cross correlation analyses of habitat characteristics associated with hosts and vectors. Cross-correlation examines a data set for correlations among time series at different time lags and provides the strength of the correlation (R^2) at each time lag. For this study, habitat data was available on a weekly basis from the beginning of June to early October of 2010 through 2012. I examined correlations between habitats used by robins in a given week and habitats associated with elevated *Culex* infection rates one week later through ten weeks later. I report the largest correlation between habitats as well as the time lag the correlation occurs at for each week from the beginning of June through the end of September. The correlation value provides a measure of similarity between habitats used by robins and infected *Culex* and the time lag provides the length of time between when robins used a habitat and when elevated *Culex* infection rates were observed in similar habitats.

RESULTS

Overall Habitat Selection

Robins exhibited significant nocturnal habitat selection throughout the entire summer (χ^2 = 43.6, df = 14, $p < 0.001$, Figure 4). Areas of elevated *Culex* abundances were significantly associated with certain habitats over others (χ^2 = 26.0, df = 14, $p = 0.02$, Figure 5), however elevated *Culex* MIR was not associated with specific habitat types (χ^2 = 1.47, df = 14, $p = 0.99$, Figure 6).

Robins over utilized wooded areas, areas near natural water, and areas with other natural cover as nocturnal habitats, while avoiding habitats near schools, apartment complexes or other buildings (Figure 4, Table 1). At the same time, robins used habitats in proximity to single family homes (houses, garages, and lawns, driveways and sidewalks) similarly to their

availability in the landscape (Figure 4).

Elevated *Culex* abundances were associated with wooded areas, natural water, grass, and natural cover, and suggested avoidance of residential areas (houses, garages, lawns driveways and sidewalks), and other development (apartments, schools, other buildings, Figure 5). Elevated infection rates in *Culex* vectors were not significantly associated with any habitat type (Figure 6).

Comparisons of Patterns in Habitat Selection

Since areas of elevated *Culex* infection rates showed no associations with specific habitat types, I only compared patterns of habitat selection between robins and areas of elevated *Culex* abundance. Comparisons of standardized selection indices (d_i values) indicate different patterns of habitat selection between hosts and areas of elevated vector abundance (χ^2 = 1135.26, df = 13, $p < 0.001$).

Time Lagged Associations Between Habitat Characteristics

Characteristics of habitats used by robins during the second week of June (CDC week 23), are strongly correlated (R^2 = 0.54, Table 4) to habitats characteristics of areas where *Culex* infection rates are elevated four weeks later during the first week of July (CDC week 27). Habitat use by robins during the third week in June (CDC week 24) was not significantly correlated with characteristics of areas with elevated *Culex* infection rates at later time periods (R^2 = 0.28, Table 4). Beginning in the fourth week of June (CDC week 25), however, habitat characteristics of areas used by birds is again moderately correlated to habitats associated with elevated *Culex* infection rates four weeks later in mid-July (CDC week 29, R^2 = 0.42). The habitat characteristics of areas used by birds in the last week of June and first week of July (CDC week 27) are strongly correlated to characteristics of areas with elevated *Culex* infection at the

beginning of August (CDC week 31, $R^2 = 0.51$). During the second week of July (CDC week 28), habitats used by robins were strongly correlated to characteristics associated with elevated *Culex* infection rates during the second week of August (CDC week 32, $R^2 = 0.57$, Table 4). Between the third week in July (CDC week 29) and the second week in August (CDC week 32), correlations between habitat characteristics associated with robins and infected vectors are weak, with the exception of the last week in July (CDC week 30), during which habitats used by robins correlated moderately to habitats associated with *Culex* infection rates four weeks later in late August (CDC week 34, $R^2 = 0.39$, Table 4). In the third week of August (CDC week 33), habitats used by birds were correlated with habitats associated with elevated *Culex* infection rates two weeks later at the beginning of September (CDC week 35, $R^2 = 0.56$), and this correlation continues in the last week of August (CDC week 34), when habitat use by birds reaches its strongest correlation to areas of elevated *Culex* abundance two weeks later (CDC week 36, $R^2 = 0.63$).

DISCUSSION

My results support the hypothesis that vector-host interactions leading to WNV transmission is not driven by temporally coincident habitat selection by vectors and hosts in the Chicago system. Patterns of habitat selection by *Culex* vectors and robins differed, and there were no significant associations between specific habitat types and areas of elevated vector infection rates. Based on this, it appears that

Cross-correlation of habitat characteristics associated with robins and vector infection rates in this system support temporal variation in the importance of robins to the transmission cycle of WNV. More specifically, the evidence for early season and late season vector-host interactions suggests the presence of robins early in the season influences WNV transmission

later, but also that the presence of robins late in the transmission season may further potentiate outbreaks of WNV in the Chicago system.

Comparison of habitat selection indices indicated *Culex* and robins selected habitats differently in the Chicago system, despite similar habitats being selected. While both vectors and hosts utilized wooded areas disproportionately, the selection index for robins was much higher than for *Culex*. Similarly, while both groups selected areas near natural water, *Culex* showed much stronger preference for natural water than did robins. In context of the breeding biology of *Culex* and the behavioral ecology of robins, these results make sense. *Culex* mosquitoes utilize stagnant water as breeding habitats (Hamer et al. 2014; Ruiz et al. 2010) and as such, habitats near water sources are likely to harbor larger numbers of *Culex*. The importance of water to the breeding biology of *Culex* would also explain the high selection index of *Culex* for areas near retention basins (Figure 5), although the selection index for this habitat class was non-significant.

For robins, the selection of wooded areas as nocturnal habitat is also consistent with nocturnal behaviors of most bird; roosting in wooded areas provides protection from weather conditions and predators (Eiserer 1984; Walsberg and King 1980). Robins are known to form large communal roosts in wooded areas or areas with dense cover as well (Brewster 1890; Howell 1942), which would account for their significant selection of areas classified as natural cover (Figure 4). I identified several large communal robin roosts throughout the study area, and many tracked birds consistently made use of communal roosts throughout the study. Communal roosting habitat likely resulted in non-independent habitat selection by some robins. At the same time, individual robins exhibit highly variable roosting behavior from night to night (Benson et al. 2012), and I tagged nestling and young individuals at developmental stages too young to fly

to communal roosts (Howell 1942). Furthermore, communal roosting has been documented in robins for over 100 years throughout North America (Brewster 1890), indicating this behavior likely serves an important. As such, suitable habitat for communal roosts is likely an important resource for robins, which would mean large selection index values for such areas would make sense in terms of robin biology.

The temporal shifts in the correlations between habitat use by robins and areas associated with elevated Culex infection rates are a unique finding to this study. While habitat use by robins in the first two weeks of July is strongly correlated with habitat characteristics of areas with elevated Culex infection rates in the first and second weeks of August ($R^2 = 0.51$, $R^2 = 0.57$), the strength of the correlation drops sharply after this point for several weeks (Table 4). During the third week of August (CDC week 32), the correlation becomes significant again for two weeks ($R^2 = 0.56$, $R^2 = 0.63$), but at a lag of only two weeks instead of four (Table 4). The time frame of non-significant habitat correlations coincides with the timing of declining blood meals derived from robins in the Chicago system and elsewhere (Hamer et al. 2008; Hamer et al. 2011; Kent et al. 2009); however no previous study has found support for the presence of robins late in the season influencing the course of WNV outbreaks. In terms of robin behavior, these findings are consistent with patterns of robin dispersal and aggregation prior to migration (Benson et al. 2012).

Consistent with the results of this study, previous work suggests environmental conditions or land-use practices associated with co-occurrence of vectors and important host species leads to increased transmission of arthropod-borne viruses, or arboviruses, such as WNV (Chase and Knight 2003; Shaman et al. 2002; Crowder et al. 2013; Reisen 2010). A study of St. Louis encephalitis (SLE) transmission in Florida suggests spatial co-occurrence of vectors and

hosts during drought periods may lead to outbreaks of SLE by facilitating vector-host interactions (Shaman et al 2002). Similarly, land use associated with elevated *Culex* and robin densities was also associated with increased WNV prevalence in *Culex* vectors and WNV transmission to horses (Crowder et al. 2013). At the same time, the finding of elevated WNV prevalence in vectors in relation to agricultural land use was not associated with increased WNV transmission to humans (Crowder et al. 2013). *Culex* feeding preferences vary geographically and have been associated with genetic differences among populations of *Culex* (Huang et al. 2009; Kilpatrick et al. 2007), which may explain the lack of transmission to humans associated with elevated vector infection rates observed in other studies. Furthermore, the main vectors of WNV vary geographically across North America, and local abundances of different species or subspecies of *Culex* varies with land cover (Bowden, Magori and Drake 2011). Due to significant geographic variations among *Culex* populations in habitat associations and feeding preferences, habitat associations of key vector species must consider genetic variation in vector feeding preferences as well. *Culex* vectors in Chicago are known to take blood meals from humans (Hamer et al. 2009; Huang et al. 2009); therefore, interactions between robins and *Culex* leading to elevated infection rates in *Culex* late in the season may lead to WNV transmission to humans.

While this study focused on generalized patterns of habitat use by vectors and hosts across years, the significant variation in WNV transmission among years may indicate differences in habitat use and overlap between vectors and hosts mediated by weather conditions. The incubation time of WNV in *Culex* vectors is highly dependent on temperature in laboratory studies (Kilpatrick et al. 2008; Reisen et al. 2006). Similarly, warmer temperatures correlated with elevated MIR in field collected *Culex*, suggesting temperature mediated effects on infection

in vectors under natural conditions (Ruiz et al. 2010). Despite the importance of temperature to infection rates in mosquitoes, it is unclear how interactions between hosts and vectors may shift under different climatic conditions. Future work should address how weather impacts habitat selection by hosts, since habitat selection by birds may be an important driver determining where vectors and hosts interact (Crowder et al. 2013).

Early interactions between *Culex* and robins may lead to transmission of WNV early in the season, but this transmission does not become apparent in vectors until later in the summer due to the incubation period of WNV within vectors , which may last anywhere from a few days to a several weeks (Kilpatrick et al. 2008). Since robins occur at high breeding densities in the Chicago suburbs (Loss et al. 2009) it is unlikely robin abundance in suburban areas limits WNV transmission early in the season. Increasing evidence suggests overwintering female mosquitoes in diapause are responsible for the seasonal reemergence of WNV (Nelms et al. 2013a; Nelms 2013b; Reisen et al. 2010), and the interaction between habitats associated with overwintering *Culex* vectors may determine where early season transmission between *Culex* and robins occur. Clarifying habitats which are used by overwintering adult mosquitoes may therefore provide a useful focus for early season control efforts to reduce the intensity of WNV outbreaks late in the season.

The correlational nature of my results is limitation of this study. Although habitat composition and fragmentation of areas used by robins early in the season correlated significantly with habitat composition and fragmentation associated with areas of elevated *Culex* infection rates later in the season, similar landscape characteristics do not mean elevated *Culex* infection rates occur exactly where robins were early in the season. As mentioned previously, robins occur at high densities throughout the Chicago suburbs, making it unlikely that robins are

a limiting factor for WNV transmission. Another possible explanation of my findings may be that habitats used by robins early in the season are also used extensively by other birds. Presence of other birds in similar habitats could provide blood meal sources for *Culex* vectors even after robins have dispersed, however this explanation would not adequately explain the disproportionate contribution of robins to the transmission cycle of WNV previously established for the Chicago suburbs (Hamer et al. 2011). Alternatively, it may not be possible to make meaningful distinctions about habitat use by birds or mosquitoes based on the habitat classes as digitized from orthophotos. Given that the habitat selection indices for vector and host which were calculated from the same habitat classes made biological sense for *Culex* and robins in terms of ecology and behavior, it appears the fifteen habitat classes used were useful for answering the questions asked in this study.

FIGURES AND TABLES

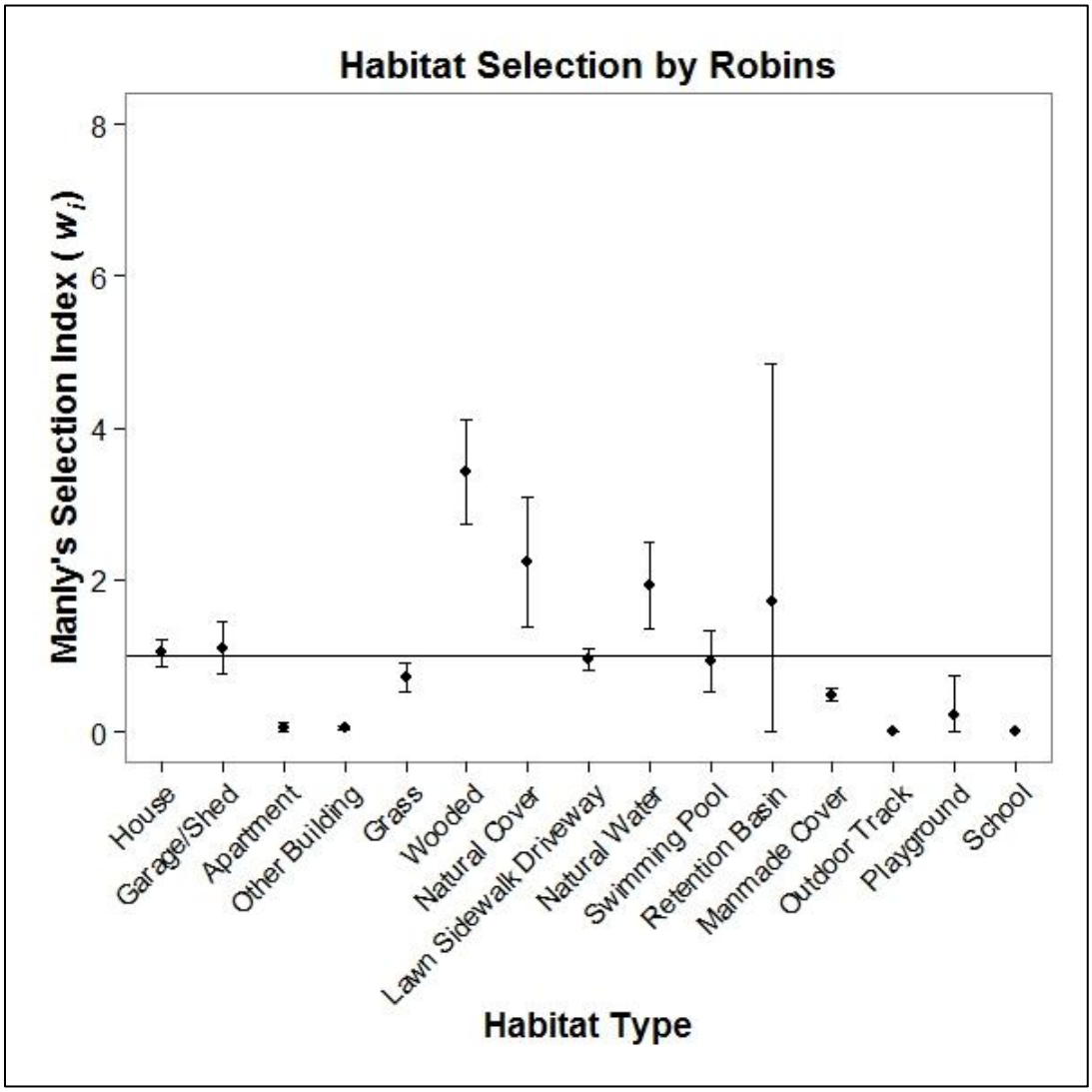

Figure 4. Manly's selection indices for habitat classes selected by robins across all weeks, +/- 95% confidence intervals. Confidence intervals entirely above or below the horizontal line are significant at the $p < 0.05$ level.

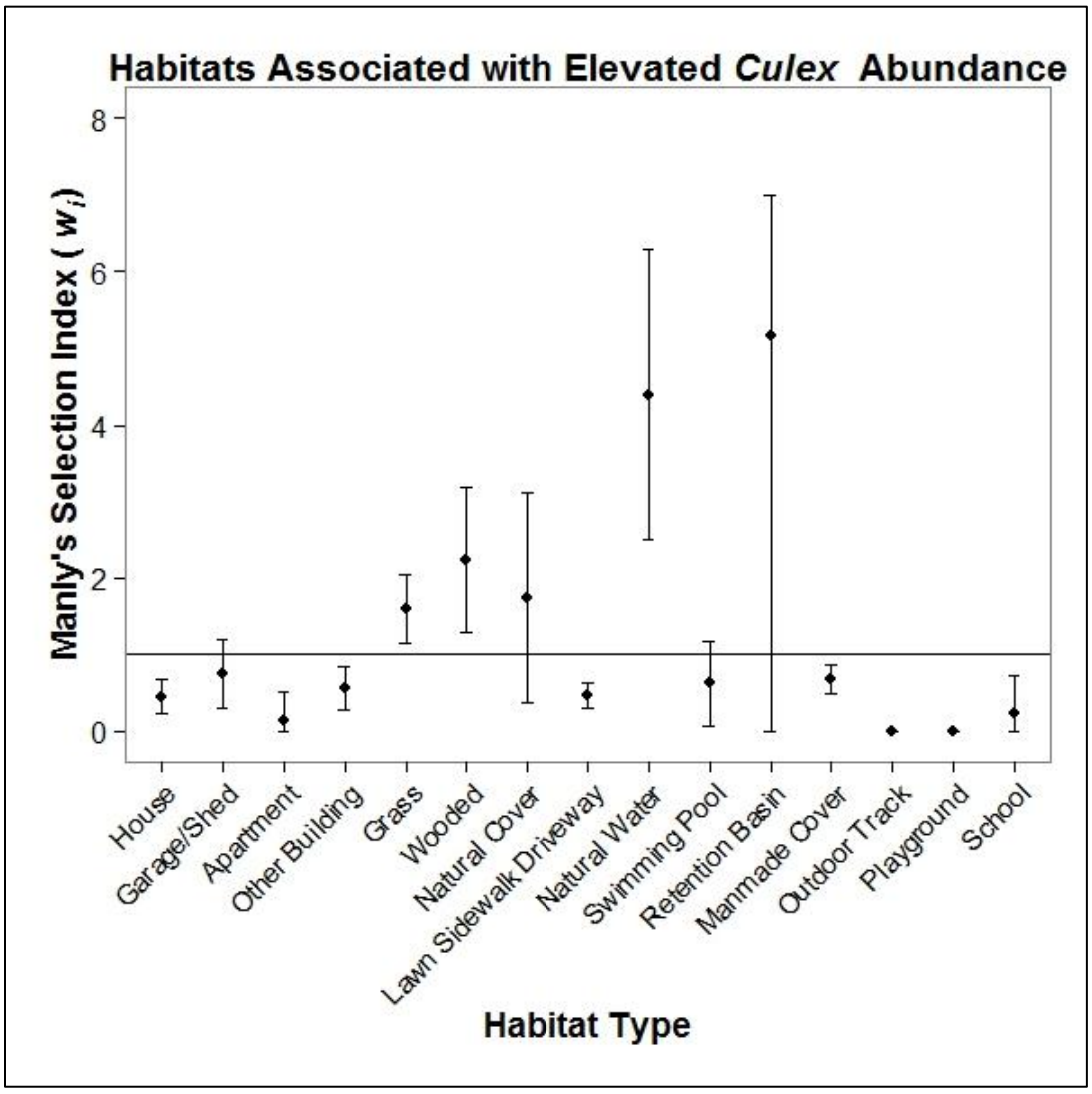

Figure 5. Manly's selection indices for habitat classes associated with elevated *Culex* abundance across all weeks, +/- 95% confidence intervals. Confidence intervals entirely above or below the horizontal line are significant at the $p < 0.05$ level.

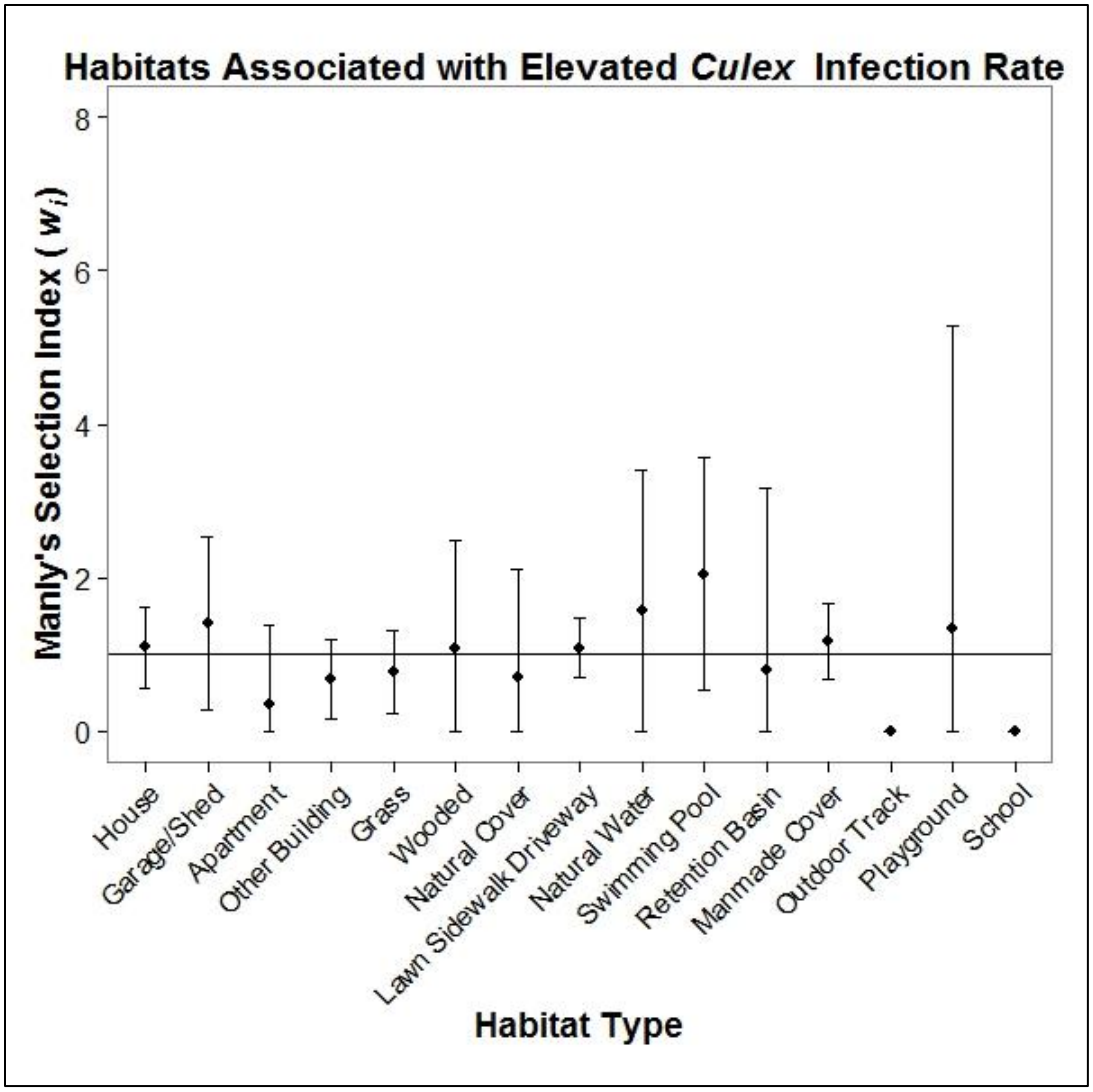

Figure 6. Manly's selection indices for habitat classes associated with elevated *Culex* MIR across all weeks, +/- 95% confidence intervals. Confidence intervals entirely above or below the horizontal line are significant at the $p < 0.05$ level.

Table 1. Pairwise comparisons of habitat selection indices among classes for robins, + or – indicates significant selection or avoidance at the $p < 0.05$ level. Comparisons are row to column, e.g. row labeled "School" to column labeled "House" indicates significant avoidances of schools compared to houses.

Comparisons of Manly's Selectivity Ratios (w_i) Among Habitat Classes Selected by Robins

Habitat Class	House	Garage or Shed	School	Apartment	Other Building	Grass Area	Wooded	Other Natural Cover	Lawn Sidewalk Driveway	Natural Water	Swimming Pool	Retention Basin	Manmade Cover	Playground	Outdoor Track
House															
Garage or Shed															
School	-	-													
Apartment	-	-													
Other Building	-	-													
Grass Area			+	+	+										
Wooded	+	+	+	+	+	+									
Other Natural Cover	+		+	+	+	+									
Lawn Sidewalk Driveway			+	+	+		-	-							
Natural Water	+		+	+	+	+	-		+						
Swimming Pool			+	+	+		-	-		-					
Retention Basin											+				
Manmade Cover	-	-	+	+	+		-	-	-	-					
Playground	-	-					-	-	-	-					
Outdoor Track	-	-			-	-	-	-	-	-	-		-		

Table 2. Pairwise comparisons of habitat selection indices among classes for elevated *Culex* abundance, + or – indicates significant selection or avoidance at the $p < 0.05$ level. Comparisons are row to column, e.g. row labeled "School" to column labeled "House" indicates significant avoidances of schools compared to houses.

Comparisons of Manly's Selectivity Ratios (w_i) Among Habitat Classes Associated with Elevated *Culex* Abundance

Habitat Class	House	Garage or Shed	School	Apartment	Other Building	Grass Area	Wooded	Other Natural Cover	Lawn Sidewalk Driveway	Natural Water	Swimming Pool	Retention Basin	Manmade Cover	Playground	Outdoor Track
House															
Garage or Shed															
School															
Apartment															
Other Building															
Grass Area	+		+	+	+										
Wooded	+	+	+	+	+										
Other Natural Cover															
Lawn Sidewalk Driveway						-	-								
Natural Water	+	+	+	+	+	+			+						
Swimming Pool							-			-					
Retention Basin															
Manmade Cover				+		-	-			-					
Playground	-	-			-	-	-	-	-	-			-		
Outdoor Track	-	-			-	-	-	-	-	-			-		

Table 3. Pairwise comparisons of habitat selection indices among classes for areas of elevated *Culex* MIR, + or – indicates significant selection or avoidance at the $p < 0.05$ level. Comparisons are row to column, e.g. row labeled "School" to column labeled "House" indicates significant avoidances of schools compared to houses.

Comparisons of Manly's Selectivity Ratios (w_i) Among Habitat Classes Associated with Elevated *Culex* Minimum Infection Rate

Habitat Class	House	Garage or Shed	School	Apartment	Other Building	Grass Area	Wooded	Other Natural Cover	Lawn Sidewalk Driveway	Natural Water	Swimming Pool	Retention Basin	Manmade Cover	Playground	Outdoor Track
House															
Garage or Shed															
School	-	-													
Apartment															
Other Building			+												
Grass Area			+												
Wooded															
Other Natural Cover															
Lawn Sidewalk Driveway			+												
Natural Water															
Swimming Pool			+												
Retention Basin															
Manmade Cover			+												
Playground															
Outdoor Track	-	-			-	-			-		-		-		

Table 4. Cross-correlation results comparing habitats used by birds and habitats associated with elevated *Culex* MIR later in the season

Time Lagged Correlations Between Characteristics of Habitats Used by Birds and Habitats Associated with Elevated *Culex* MIR

Bird Week	*Culex* MIR Week	Lag in Weeks	Correlation
23	27	4	0.54*
24	32	8	0.28
25	29	4	0.42*
27	31	4	0.51*
28	32	4	0.57*
29	37	8	0.22
30	34	4	0.39*
31	39	8	0.24
32	34	2	0.25
33	35	2	0.56*
34	36	2	0.63*
35	37	2	0.41*
36	37	1	0.27
37	38	1	0.28
38	40	2	0.31

* Significant at $p < 0.05$ level

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CHAPTER 4: PARASITISM AND IMMUNE FUNCTION IN NESTLING AMERICAN ROBINS

ABSTRACT

Life-history theory predicts organisms face trade-offs in how they allocate limited energetic resource to various life-history tasks, such as growth, reproduction, or immune function. Studies of trade-offs in immune function tend to focus on Passerine birds due to energetic constraints associated with rapid periods of growth during the nestling phase, and increasingly evidence suggests nestling birds face trade-offs between growth and investment in immune function during this period. Exposure to external parasites during the nestling phase can stimulate nestlings to invest in immune function over growth; however whether internal parasites can have a similar effect is less clear. Internal parasites such as helminths can over activate parts of a host's immune system at the expense of other branches of immunity. Thus, infection with helminths can cause increased susceptibility to other pathogens or parasites in a host. Therefore, infection by helminthes may suppress immune function or increase investment in immunity in nestling birds. I tested the hypothesis that infection by helminths shapes how nestling American robins (*Turdus migratorius*) invest in immune function during the nestling period. I treated experimental nestlings with a broad-spectrum ant-helminthic drug and estimated white blood cells concentrations before and after treatment for parasites. I found no difference in white blood cell concentrations or body condition between treated and untreated nestlings. Thus, it appears that at least in American robins, early exposure to parasites does not influence how nestlings invest energy into immune development.

INTRODUCTION

Historically, studies of the vertebrate immune system have sought to describe the action of the immune system under controlled conditions (Sadd and Schmid-Hempel 2009), however increasing interest in how organisms allocate resources to immune function to maximize fitness in natural settings has led to the emergence of eco-immunology and evolutionary immunology as unique fields of study (Martin et al. 2011). Eco-immunologists are interested in how organisms invest in immune function to maximize fitness while maintaining plasticity in immune response under changing ecological, physiological and nutritional constraints (Wikel, et al. 1994; Weiss and Aksoy 2011; Maule et al. 1989; Wodarz 2006).

Due to nutritional and energetic constraints, organisms face trade-offs in how they allocate energy and nutrients to different life-history tasks such as growth, reproduction, or immune function (Lochmiller and Deerenberg 2000; Zuk and Stoehr 2002; Sheldon and Verhulst 1996; Svensson et al. 1998). For example, nestling birds face trade-offs between immune function and growth (Soler et al. 2003; Tschirren et al. 2006), and many species decrease reproductive effort in response to immune challenges during breeding or vice versa (Hanssen et al. 2005; de Lope et al. 1998; French et al. 2007; Deerenberg et al. 1997). Trade-offs between immunity and other life history tasks can have significant fitness impacts due to differences in survival or reproduction. In nestling birds, body size at fledging is positively correlated with post-fledging survival in birds (Both et al. 1999), and across taxa the fitness gained from current reproductive effort must be balanced against fitness gained by surviving to reproduce again (French et al. 2007; Hanssen et al. 2005). Thus, organisms should balance investment in immune function against other imminent life history tasks.

In addition to energetic trade-offs between immune function and other life-history tasks,

organisms face trade-offs between investment in different components of the immune system (Forsman et al. 2008; Jolles et al. 2008). The vertebrate immune system consists of innate and adaptive components (Tizard 2012). While the innate immune system provides general protection against infection with inflammatory responses or complement proteins, the adaptive immune system is capable of 'remembering' infectious agents previously encountered by an organism and providing lasting immunity (Tizard 2012). The adaptive immune system can also mount specific modes of response depending on the nature of an invading parasite. Intracellular infections, such as viruses, elicit a T-helper 1 (Th1) response while extra-cellular parasites, such as helminths, are mediated by a T-helper 2 (Th2) response (Hawley and Altizer 2011; Romagnani 1997; Romagnani 2000).

Although the causative agent of an infection determines whether a host mounts a Th1 or Th2 response, interactions between parasites and the host's immune system are complex. Infection by parasites can stimulate or suppress host immune responses, depending on the parasite or the age at which a host becomes infected with a parasite. For example, ecto-parasitism has been shown to stimulate investment in both adaptive and innate immune function by nestling songbirds (Owen et al. 2010; Brommer et al 2011; Tschirren et al. 2009; Tschirren et al. 2007; Tschirren and Richner 2006; Schoeler and Wikel 2001), and early exposure to parasites can influence long-term patterns of immune response in mammals as well as birds (Cooper et al. 2001; Steel et al. 1994; Tschirren et al. 2007). Conversely, infection by helminths can suppress the Th1 immune response while over-activating the Th2 response in a phenomenon known as immune polarization, which can result in increased susceptibility of the host to Th1 mediated infections (Helmby 2009; Girgis, et al. 2013; Stewart et al. 1999; Ezenwa et al. 2010; Jolles et al. 2008). Whereas immune polarization due to helminth infection is well documented in mammals

(Ezenwa et al. 2010; Helmby 2009; Stewart et al. 1999), it has only recently been identified in birds (Degen et al. 2005).

The recent discovery of immune polarization in birds has particular significance for eco-immunology. Much of the available information regarding the ontogeny of the vertebrate immune system was established using domestic chickens (*Gallus gallus domesticus*) under laboratory conditions (Degen et al. 2005; Martin et al. 2011), meaning the current understanding of immune development does not consider interactions between parasitism and the development of immune responses in young organisms. Furthermore, eco-immunologists frequently use nestling birds to study the ontogeny of immune function or life history tradeoffs between immunity and other traits in natural systems due to energetic constraints associated with rapid growth during the nestling period (Tinbergen and Boerlijst 1990; Both et al. 1999). Despite evidence for increased investment in immune function by nestling birds fed on by ecto-parasites (Tschirren et al. 2006) and support for long-term patterns of immune function shaped by early exposure to parasitism, how early exposure to internal parasites influences immune development in nestling songbirds has not been previously studied. Understanding whether parasitism stimulates or suppresses immune development in young organisms is a central question to eco-immunology, and may have important implications for transmission of diseases in wild populations (Ezenwa et al. 2010; Vandegrift et al. 2010; Friberg et al. 2010; Girard et al. 2011).

To test whether infection by internal parasites leads to investment in immune function over growth or results in immune polarization in nestling birds, I conducted a medication experiment in nestling American robins (*Turdus migratorius*). I treated nestling American robins with a broad-spectrum anti-parasitic drug (fenbendazole) or distilled water (as a procedural control), then took measurements relating to body condition before and after treatment as well as

blood samples to assess changes in white blood cell profiles associated with reduction of internal parasite burden. I estimated total white blood cell concentrations before and after treatment and used estimates of concentrations of eosinophils as a proxy measure of Th2 immune response to parasite burden. I also estimated concentrations of heterophils and lymphocytes, which are the most commonly circulating leukocyte in birds (Campbell and Ellis 2007). Heterophils and lymphocytes are both involved in the innate immune response, as well as the Th1 response in birds.

I hypothesized that infection with internal parasites could suppress Th1 immune function, stimulate investment in all forms of immune function, or not impact investment in immune function. If infection by helminths leads to immune polarization, then treated nestlings should have lower concentrations of heterophils and lymphocytes than control nestlings. If infection by internal parasites stimulates overall investment in immune function, then nestlings treated for parasites should have greater concentrations of all white blood cells compared to control nestlings. If internal parasites have no impact on nestling investment in immune function, then white blood cell concentrations should be similar between treated and control nestlings, but body condition of treated nestlings should be improved compared to control nestlings due to the reduction in parasite burden freeing up energetic resources for growth.

METHODS

From April through June in 2012 and 2013, I located robin nests in Urbana and Chicago, Illinois. All nests were located either during laying or early incubation, and monitored every two days until hatching occurred. Nests in both locations were located in suburban developments, and weather patterns in both locations are typically similar throughout the summer; therefore, I have no reason to believe the location of a nest influenced the exposure of nestlings to parasites

or response to treatment.

Nestling Measurements

I took three sets of morphological measurements and blood and fecal samples from nestlings, beginning on day six post-hatching. On day six, I measured body mass and tarsus length of all nestlings, took a blood sample, and banded nestlings with a unique combination of color bands to identify individuals for subsequent measurements. I weighed and measured tarsus length again on day 8 and day 10 to monitor nestling growth post-treatment.

Assignment to Treatment and Treatment Procedure

Nestlings were assigned to treatment groups randomly. On day six post-hatching I removed all nestlings from the nest and placed each in a separate handling bag. I then selected a bag randomly and assigned the first nestling selected to the treatment group, the second to the control group, third to treatment, etc. In the case of odd numbers of nestlings, I flipped a coin to determine the treatment group of the last nestling. Each nest had at least one treatment and one control nestling. I administered fenbendazole orally to treatment nestlings at a dose of 0.1 mg/kg body weight; control nestlings received a similar volume of distilled water orally as determined by body weight. On day 10, I took another blood sample to estimate differences in white blood cell counts post treatment. All animal use was approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC), protocol #12042.

` Blood samples from each nestling were of a volume of less than or equal to 0.05 mL per nestling, (approximately 0.1% of body weight by volume) using jugular venipuncture with an insulin syringe, then immediately transferred the sample to a heparinized capillary tube from which I prepared blood smears. Blood smears were air dried, and stained with Wright-Giemsa stain within a month for white blood cell counts. Paired blood sampling was unsuccessful for 18

nestlings; for these nestlings we still measured and dosed according to protocol, but nestlings without paired blood smears were excluded from hematological analyses.

Leukocyte Profiles

I examined blood smears at 1000x magnification under oil immersion to estimate total white blood cell concentration, and proportions of different while blood cell types. I counted heterophils, lymphocytes, basophils, eosinophils and monocytes following Campbell and Ellis (2007), observing at least 100 white blood cells per slide. I estimated total white blood cell counts, using proportions from white blood cell differentials to estimate total concentration per mm^3 of each white blood cell type. All blood smears were counted by a single observer, who was blind to the treatment status of each bird.

Statistical Analysis

Body condition, total white blood cell concentration, and total concentrations of heterophils, lymphocytes, and eosinophils were used as response variables for treatment of nestling in the analyses. To characterize body condition, I regressed tarsus length on body mass and derived residuals. Positive residuals are indicative of good condition and negative residuals indicate poor condition. Concentrations of basophils and monocytes were excluded from analysis, as white blood cell counts of most nestlings included 2% or less of either of these cell types. I ran repeated measures ANOVAs in program R to test for differences between treatment and control nestlings in body condition and the various concentrations of white blood cell types. I specified treatment as a fixed effect and included a random effect for individual within nest.

Identification of Helminth Infection in Nestlings

To determine whether nestlings were infected by internal parasites, I conducted sucrose fecal floatations on fecal samples from nestlings on day six post-hatching and looked for parasite

eggs or larvae (Foreyt 2002). Negative fecal floatations are not definitive evidence of lack of parasitic infection in birds due to the large volume of feces birds pass and the dilution of egg or larval concentrations (Welte and Kirkpatrick 1986). I observed parasite shedding of at least one species of unidentified helminth in samples from initial samples of three nestlings, indicating that nestling robins as young as six days post-hatching are infected. Furthermore, previous studies have found 90% of juvenile American robins were infected with at least one helminth parasite (Hamer et al. 2013), and these parasites are typically transmitted to vertebrate hosts through ingestion of arthropods containing encysted parasite larvae (Hamer et al. 2013). Young robins are likely exposed through parental provisioning of arthropods since the diet of American robins is comprised largely of arthropods during breeding (Wheelwright 1986). Upon ingestion by a host, encysted larvae can emerge from cysts within minutes to hours to infect the host (Graff and Kitzman 1965). Therefore, this experiment was conducted under the assumption that all nestling American robins are exposed to some variety of helminth larvae via ingestion during the nestling phase, and that some exposures result in infection, which is supported by the shedding of larval parasites in fecal samples of three experimental nestlings.

RESULTS

I sampled 72 nestlings at 24 nests: 20 nestlings from 7 nests in Chicago during spring and summer of 2012, 28 nestlings from 10 nests in Urbana during spring 2012, and 24 nestlings from 7 nests in Urbana in spring of 2013.

Treatment with fenbendazole had no effect on body condition ($F_{1,29} = 1.44$, $p = 0.23$). Total white blood cell concentrations were similar treatment and control birds ($F_{1,29} = 0.22$, $p = 0.64$, Figure 8A). Total heterophil concentrations in treated nestlings was not influenced by treatment (Figure 8B, $F_{1,29} = 0.30$, $p = 0.58$). The overall concentration of lymphocytes between

days 6 and 10 was not different between treatment groups (Figure 8C, $F_{1,29}$= 0.49, p = 0.48). Eosinophil concentrations were not influenced by treatment (Figure 8D, $F_{1,29}$= 0.01, p = 0.89).

DISCUSSION

The results of this study suggest infection with internal parasites has no impact on investment in immune function by nestling American robins, since there were no differences in white blood cell concentrations between treatment groups.

An unusual result observed in this study was the apparently elevated eosinophil levels of nestling robins regardless of treatment. Eosinophils in birds are expected to comprise approximately 1-5% of circulating leukocytes (Reagan 2008), but in nestlings sampled for this study comprised between 30-40% of total circulating leukocytes. Experimental infections of Herring Gull (*Larus argentatus*) chicks and red jungle fowl (*Gallus gallus*) with larvae of various helminth parasites resulted in elevated eosinophil concentrations in both experiments (Mazur et al. 2007; Johnsen and Zuk 1999), and studies of parasitized poultry have found eosinophil levels consistent with those observed in nestling American robins in this study (36% eosinophils, Maxwell and Burns 1985). Elevated eosinophil concentrations in nestling Wood Storks (*Mycteria americana*) were negatively correlated to survival during the post-fledging period (Hylton et al. 2006), indicating that underlying infections or conditions stimulating eosinophil production can have major fitness consequences. Although eosinophilia, or elevated eosinophil concentrations, can be idiopathic (Maxwell et al. 1979), or related to molt status in birds (Brake 1982; Altizer et al. 2004), eosinophilia is more frequently associated with the vertebrate immune response to parasitism (Klion and Nutman 2003; Tizard 2012). The levels and consistency of eosinophilia observed in nestling robins in this study is similar to levels observed in parasitized birds, suggesting robins are likely exposed to parasites early in life.

Therefore, robins may invest a non-trivial amount of energy in fighting or managing damage from parasitic infections over the course of their lives, suggesting parasitism is potentially an important indirect selective pressure in this species.

The lack of evidence for trade-offs between parasitism, immune function and growth in nestling robins in this study may be due to the time frame at which measurements and samples were taken from nestlings. The sampling interval may have been too short to observe differences between body condition or white blood cell counts between treated and untreated nestlings. Previous studies which support trade-offs between investment in immune function versus growth have utilized study species with nestling periods longer than that of American robins (10-14 days, Howell 1942), such as Eurasian blue tits (*Cyanistes caeruleus*, 18-21 days, Brommer 2004), Leach's Storm-Petrel (*Oceanodroma leucorhoa*, 55-65 days, Mauck, Matson, Philipsborn and Ricklefs 2005) or common magpies (*Pica pica*, 24-30 days, Soler et al. 2003), with nestlings being resampled or retreated throughout the nestling period. While all altricial birds grow rapidly during the nestling phase, the shorter nestling period of American robins may favor immediate investment in growth over immune function and longer nestling periods in other study species may allow better optimization of energetic tradeoffs between growth and immune function. A longer nestling period could be favored on an evolutionary time scale due to improved survival or fitness of more immunocompetent nestlings (Christe et al. 1998; Moller and Saino 2004), with the upper length of the nestling period limited by increased risk of nest predation the longer the nestlings remain in the nest. One study found longer incubation periods were associated with decreased cellular immune function in nestlings in a study of twelve Passerine species (Palacios and Martin 2006), however a study examining the impact of nestling period determined cellular immune function in nestlings does vary with the length of the nestling period (Tella, Scheuerlein

and Ricklefs 2002). Historically, researchers believed the ontogeny of immune development in precocial and altricial birds was similar, however recent work examining immune development in house sparrows (*Passer domesticus*) suggests this may not be the case (King et al. 2010). Given that our current understanding of avian immune function is based mainly on precocial species (King et al. 2010), the apparently lack of tradeoffs observed in this study may be more indicative of a relatively poor understanding of how life history traits of altricial birds influence when energetic tradeoffs are observable in nestlings.

The time frame between treatment and post-treatment measurements may not have been long enough to detect significant changes in white blood cell concentrations as well. Volunteer infection studies in humans found that eosinophil concentrations do not start to change until two to three weeks after infection with parasites with peak concentrations occurring within seven to nine weeks (Maxwell 1987), although experimental infections of young sheep support rapid increases in eosinophil concentrations within three days of exposure to parasites (MacKinnon et al. 2010). Experimental infections of poultry support rapid changes to white blood cell counts within several days to a week of inoculation with parasites (El-Din 2004), although peak eosinophil concentrations may not be reached until several weeks after initial exposure to parasites (Ferris and Bacha 1986) and changes in white blood cell concentrations can differ depending on the species of parasite infecting a host (Oladele et al. 2005; El-Din 2004; Ferrish and Bacha 1986). The first treatment of robin nestlings in this study was constrained by my ability to accurately measure medication doses, which was difficult before day six post-hatching due to the minute volumes of medication required by body weight of young nestlings (< 0.02 mL), as well as a lack of information on the pharmacodynamics of fenbendazole in young wild birds. Since nestling robins can fledge as early as day ten post-hatching (Howell 1942),

sampling beyond day ten increased the risk of force-fledging nestlings and restricted the number of post-treatment measurements that could be taken. White blood cell counts may have continued changing into the post-fledging period, but were simply not measured in the time frame of this study. Beyond sampling effort, single doses of fenbendazole may not have been adequate to clear parasites from the treated nestlings (Welte and Kirkpatrick 1986). Future studies of interactions between internal parasites, growth and immune function should consider the length of time available to resample nestlings post-treatment or to re-treat nestlings as appropriate while they remain in the nest.

Another possible explanation for the observed lack of interactions between parasitism, growth or immune function is treating nestlings for parasites may have freed up energy for investment in branches of the immune system not measured in this study. I attempted to measure overall immunoglobulin (IgG) concentrations from nestling blood samples using a commercially available enzyme linked immunoassay (ELISA) for IgG in chickens; however the assay either did not work on passerine IgG, was not sensitive enough for the concentrations of IgG in the nestling samples, or simply failed. It has since come to my attention that antibodies to Passerine IgG will soon be commercially available, which was not available at the time of this study. Nonetheless, treated nestlings in this study may have invested more energy into antibody production than cellular immune function. A study of nestling blue tits (*Cyanistes caeruleus*) found that experimentally inducing nestlings to invest in cellular immune function lead to reductions in circulating IgG levels, indicating investment in cellular immunity decreased investment in humoral immunity (Pitala et al. 2010). Studies of house wrens (*Troglodytes aedon*) and pied flycatchers (*Ficedula hypoleuca*) found several different measures of immune function in nestlings were not correlated (Forsman et al. 2008; Moreno et al. 2013), suggesting different

branches of the immune system can be regulated or developed independently of other branches despite significant interactions among branches of the immune system (Tizard 2012). As such, the observed lack of response of cellular immunity to treatment for parasites does not mean other branches of the immune system were not impacted. While I did not find evidence of suppressed Th1 function in nestlings not treated for parasites, there were trends indicating potentially elevated Th2 responses in the form of increasing eosinophil concentrations. I did not assay non-cellular components of innate immune function, such as complement proteins or bactericidal capacity of the blood plasma, which may provide a first line of defense against initial colonization by parasites (Tizard 2012), nor did I successfully measure antibody concentrations which are a non-cellular aspect of the Th2 immune response. While cellular measures of the Th1/Th2 immune response did not change with treatment for parasites, investment in non-cellular immunity may have been impacted. Future work should measure multiple axes of immune function while also manipulating parasite load to determine how nestling birds use parasitism to shape investment in different branches of immunity during development.

FIGURES AND TABLES

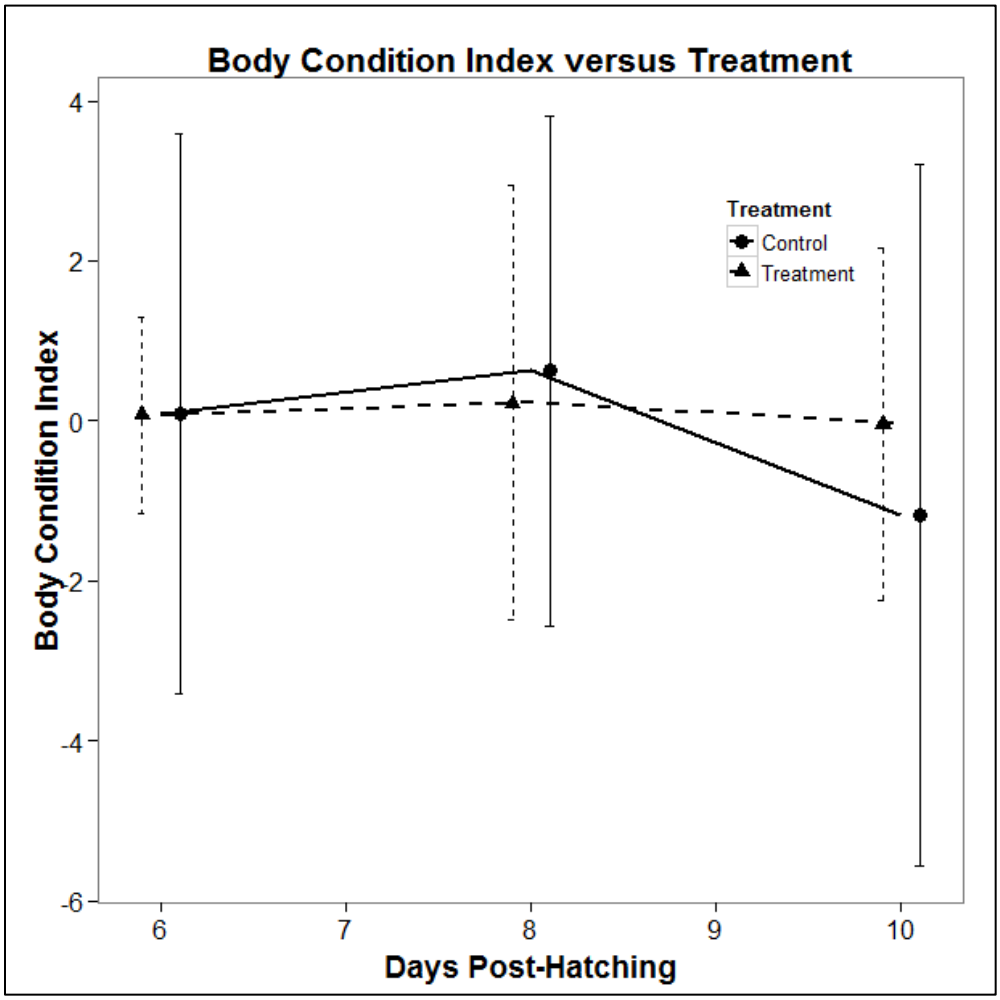

Figure 7. Changes in body condition index before and after treatment, +/- 1 standard deviation

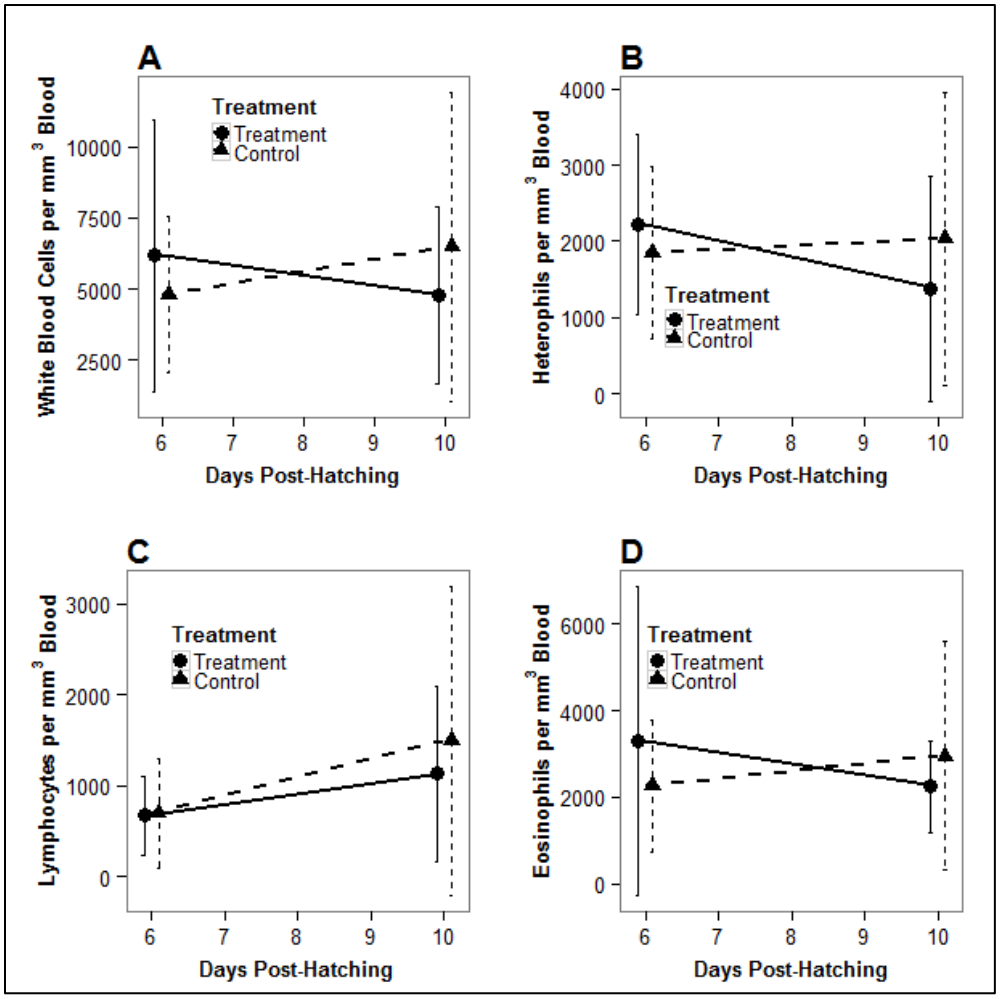

Figure 8. Changes in white blood cell concentrations between days six and ten post-hatching, +/- 1 standard deviation. All graphs are in cells per mm^3 blood. A) Total white blood cell concentrations B) Heterophil concentrations C) Lymphocyte Concentrations D) Eosinophil concentrations

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APPENDIX A: PLOTS OF CROSS-CORRELATION BETWEEN HABITAT CHARACTERISTICS ASSOCIATED WITH ROBIN USE AND MOSQUITO INFECTION RATES

Plots indicate time lag and magnitude of correlations between habitats selected by robins and habitats associated with elevated *Culex* infection rates. Time lag zero occurs at the week listed in the plot title.

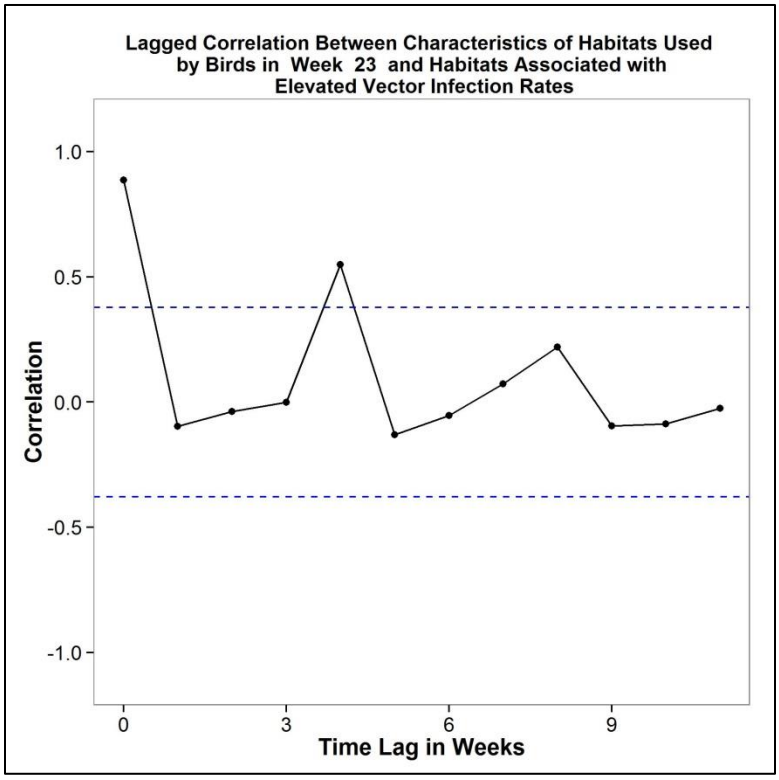

Figure 9. Cross-correlation between habitat characteristics associated with robin use during CDC week 23 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

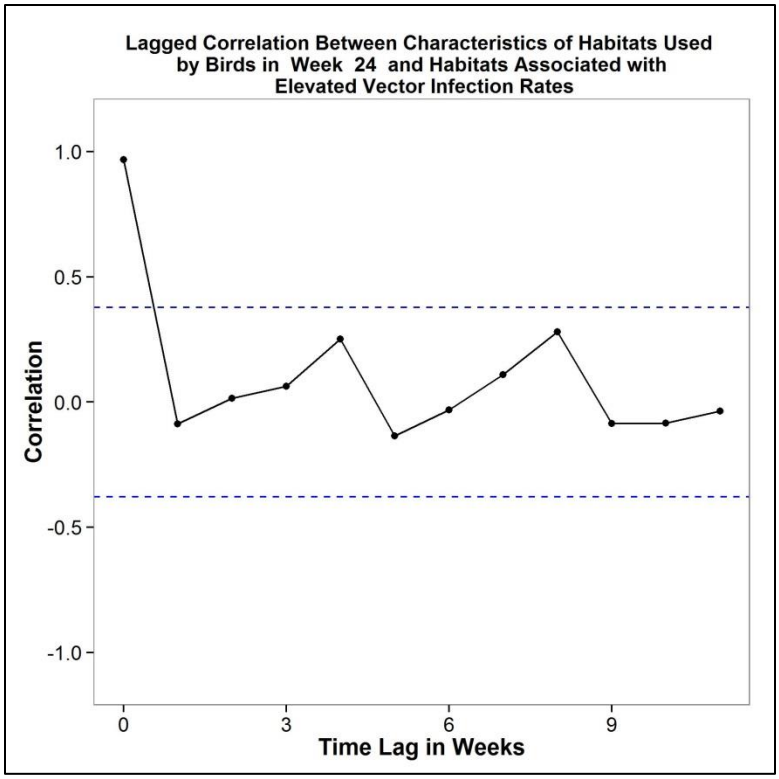

Figure 10. Cross-correlation between habitat characteristics associated with robin use during CDC week 24 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

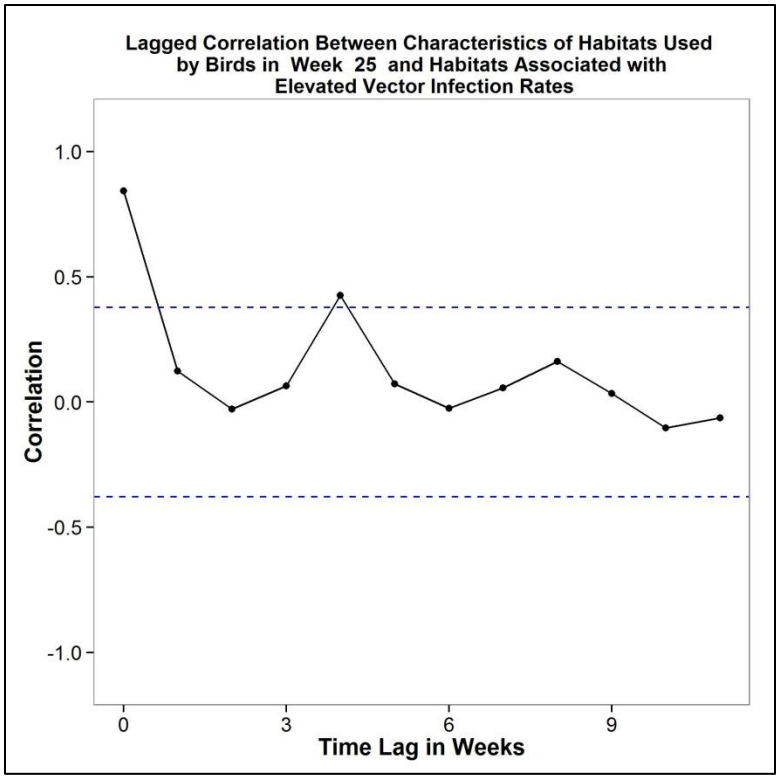

Figure 11. Cross-correlation between habitat characteristics associated with robin use during CDC week 25 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

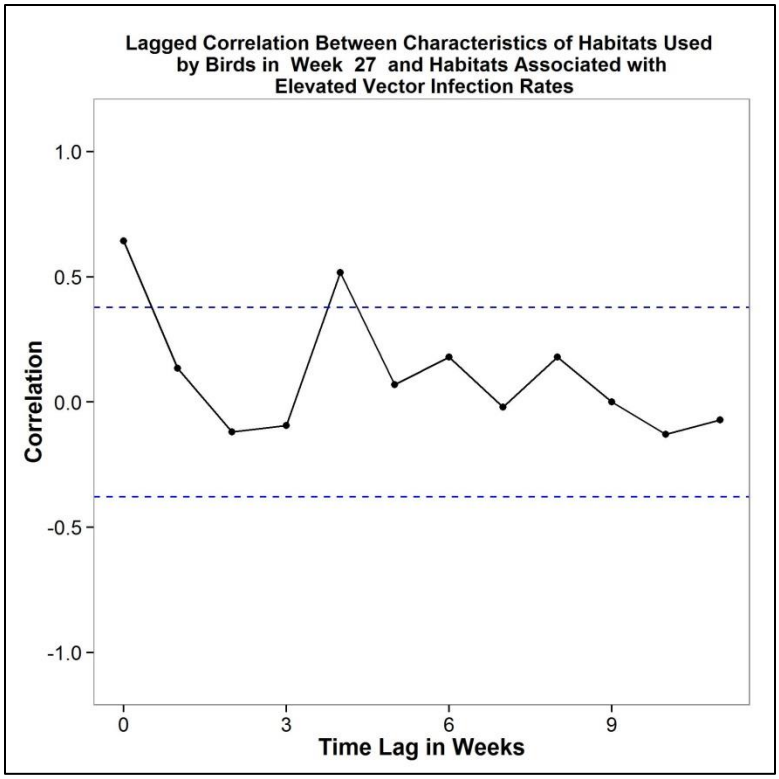

Figure 12. Cross-correlation between habitat characteristics associated with robin use during CDC week 27 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

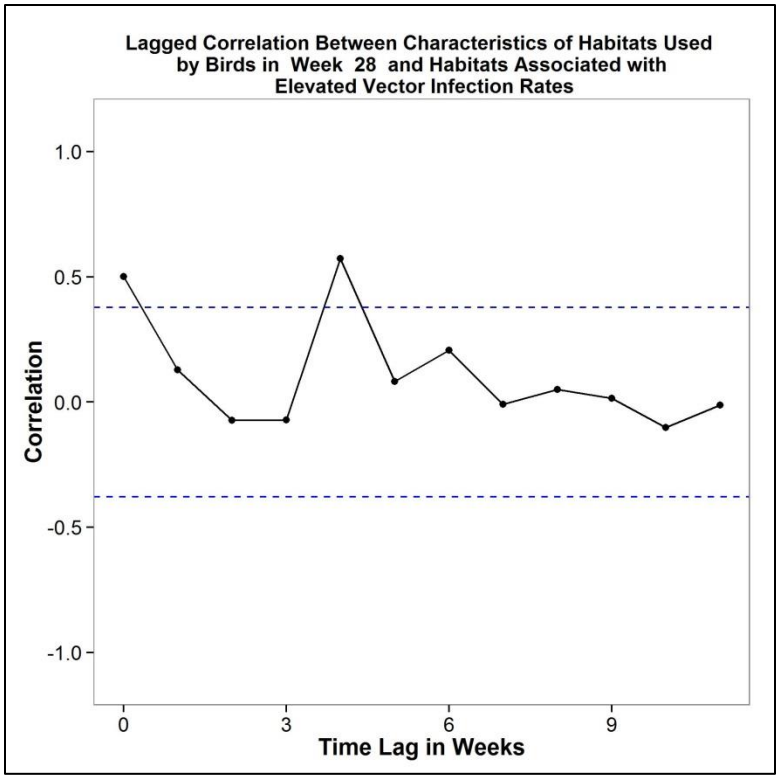

Figure 13. Cross-correlation between habitat characteristics associated with robin use during CDC week 28 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

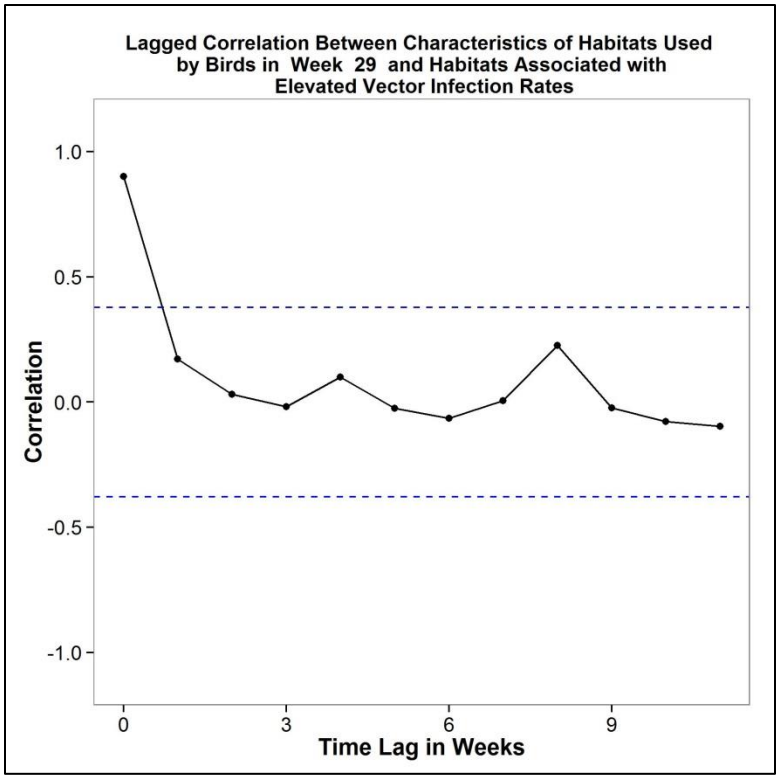

Figure 14. Cross-correlation between habitat characteristics associated with robin use during CDC week 29 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

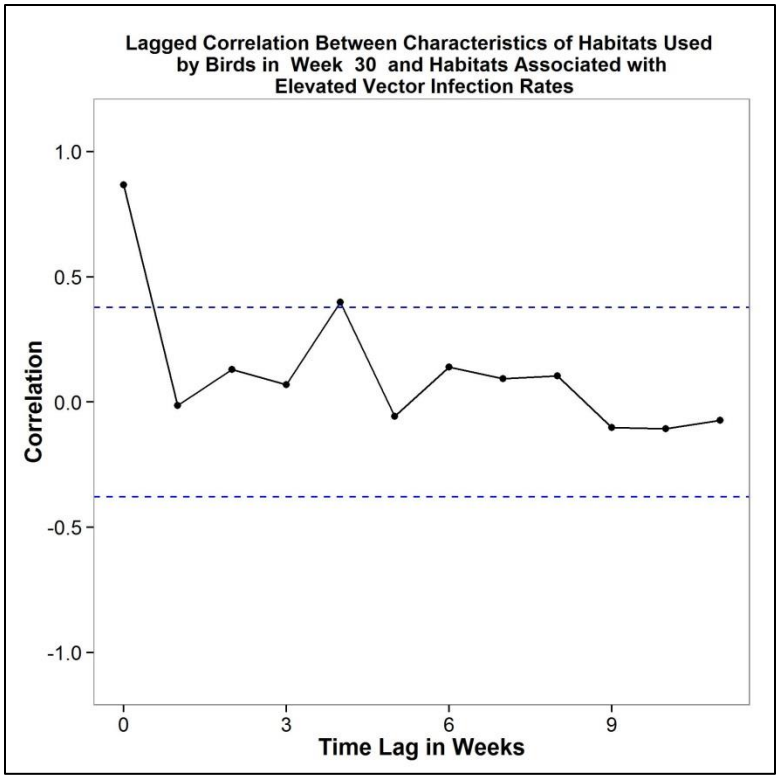

Figure 15. Cross-correlation between habitat characteristics associated with robin use during CDC week 30 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

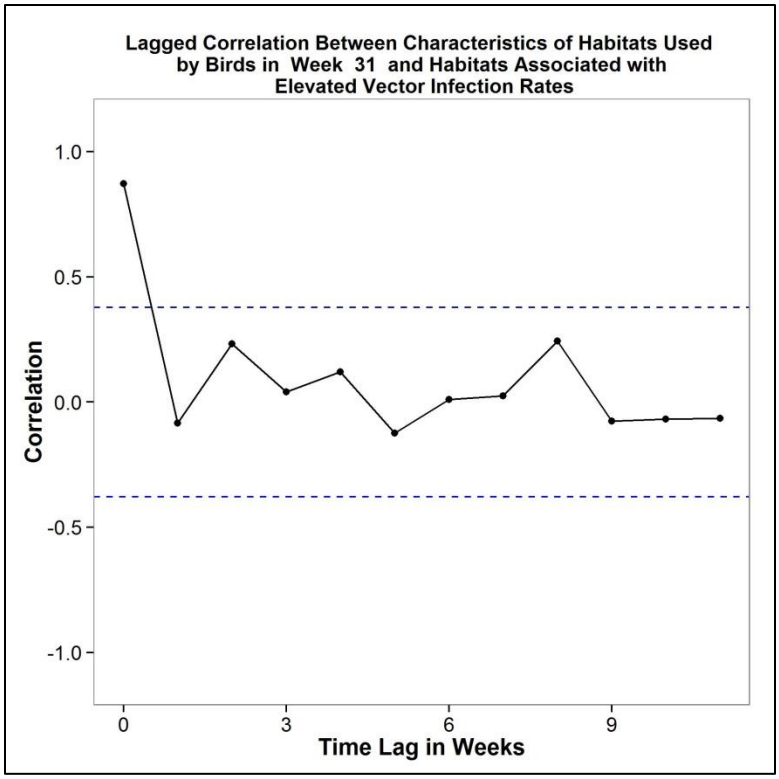

Figure 16. Cross-correlation between habitat characteristics associated with robin use during CDC week 31 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

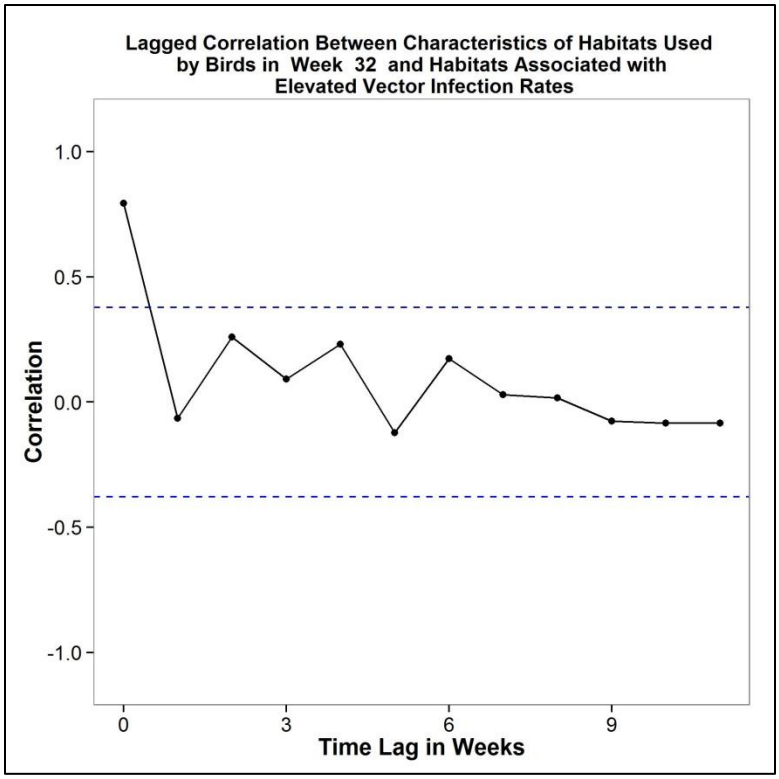

Figure 17. Cross-correlation between habitat characteristics associated with robin use during CDC week 32 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

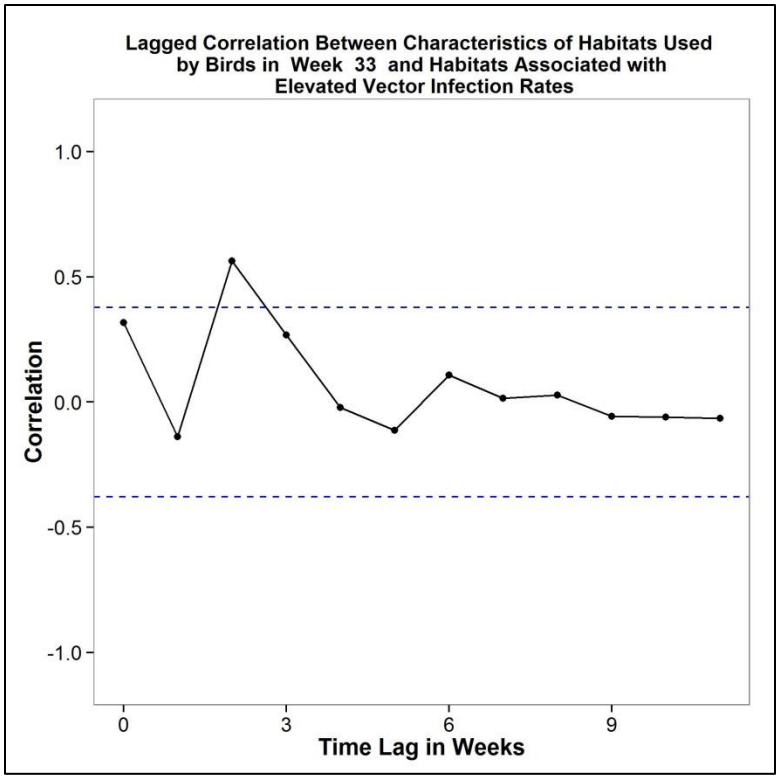

Figure 18. Cross-correlation between habitat characteristics associated with robin use during CDC week 33 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

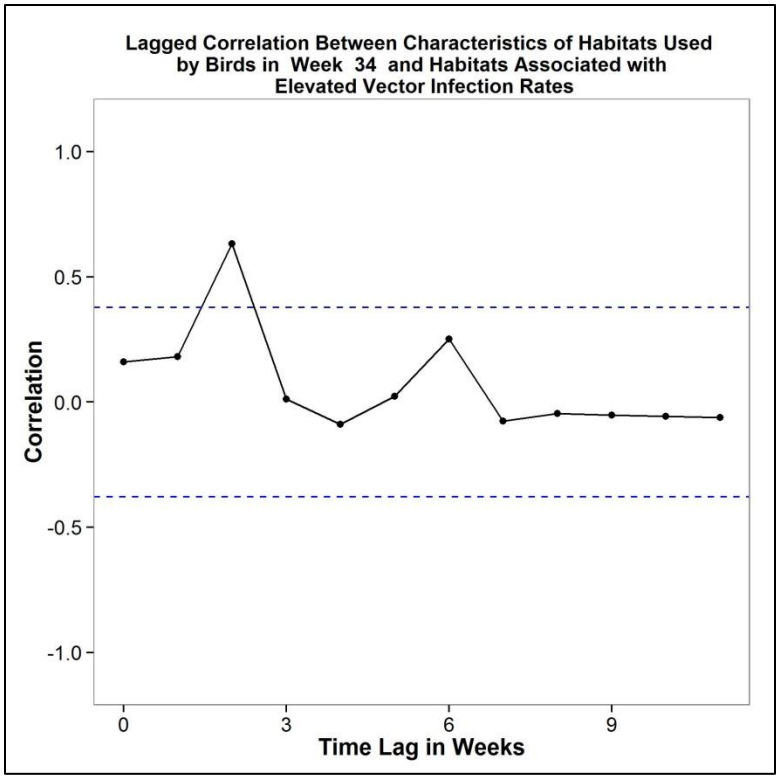

Figure 19. Cross-correlation between habitat characteristics associated with robin use during CDC week 34 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

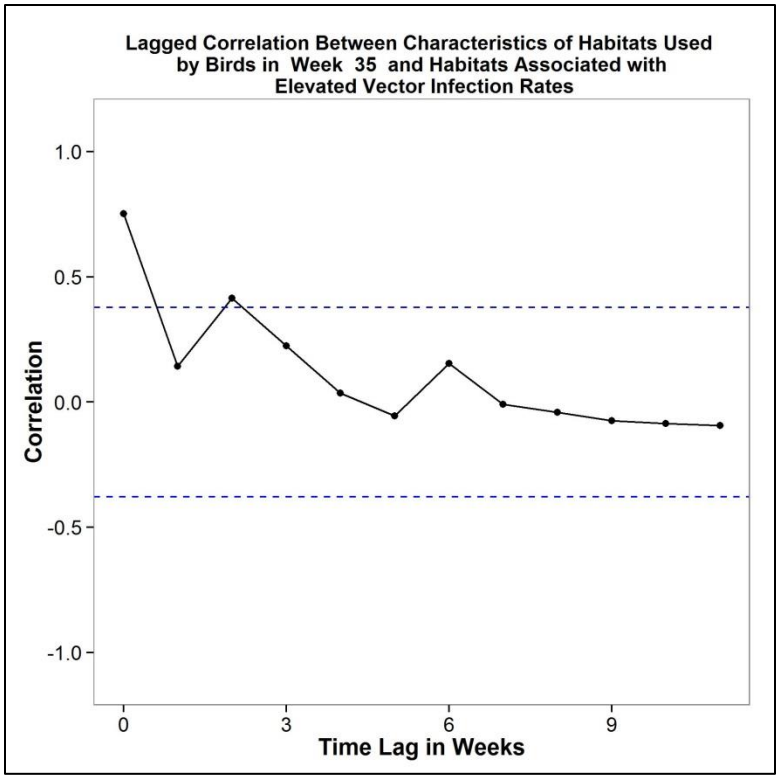

Figure 20. Cross-correlation between habitat characteristics associated with robin use during CDC week 35 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

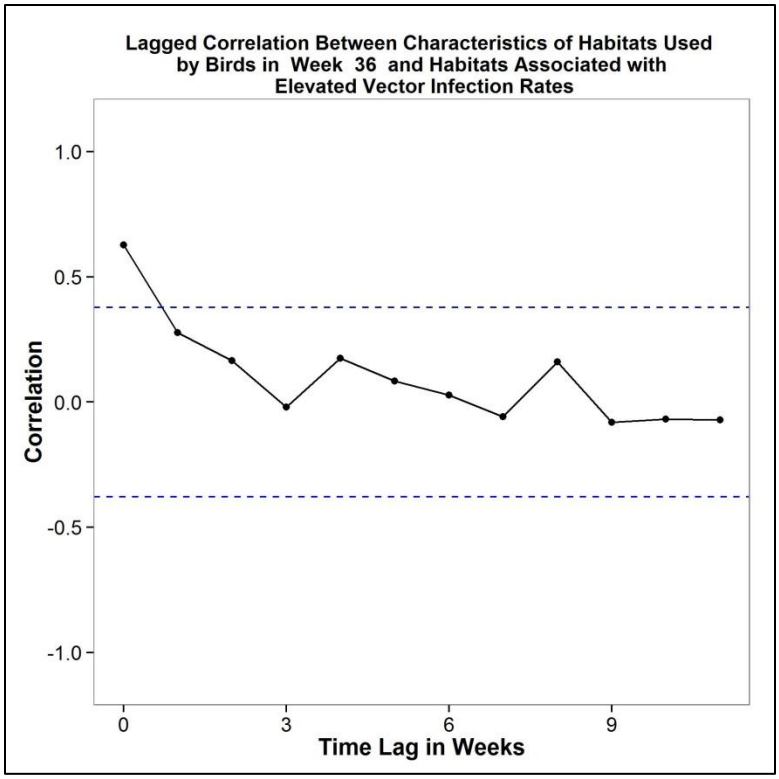

Figure 21. Cross-correlation between habitat characteristics associated with robin use during CDC week 36 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

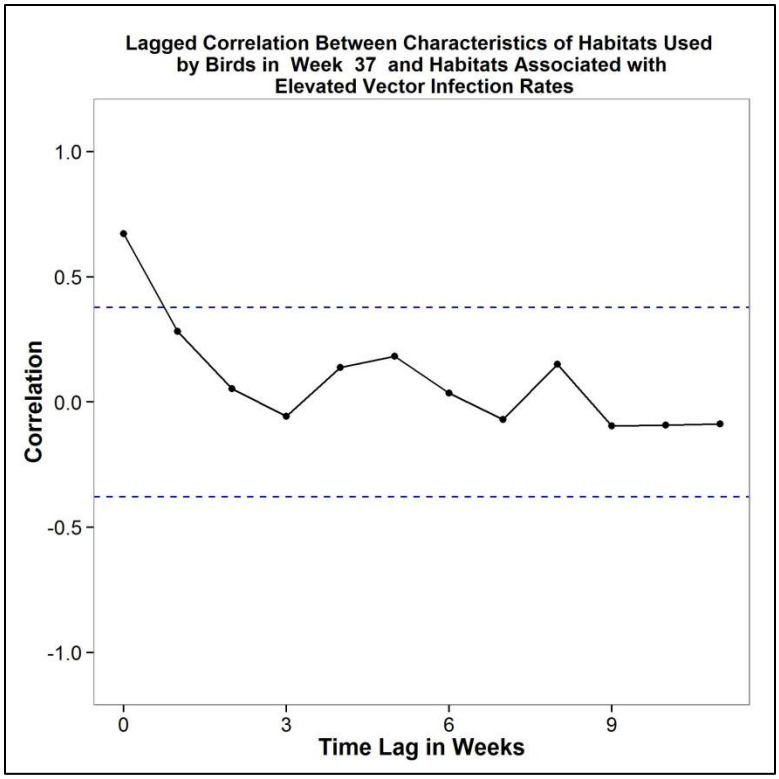

Figure 22. Cross-correlation between habitat characteristics associated with robin use during CDC week 37 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.

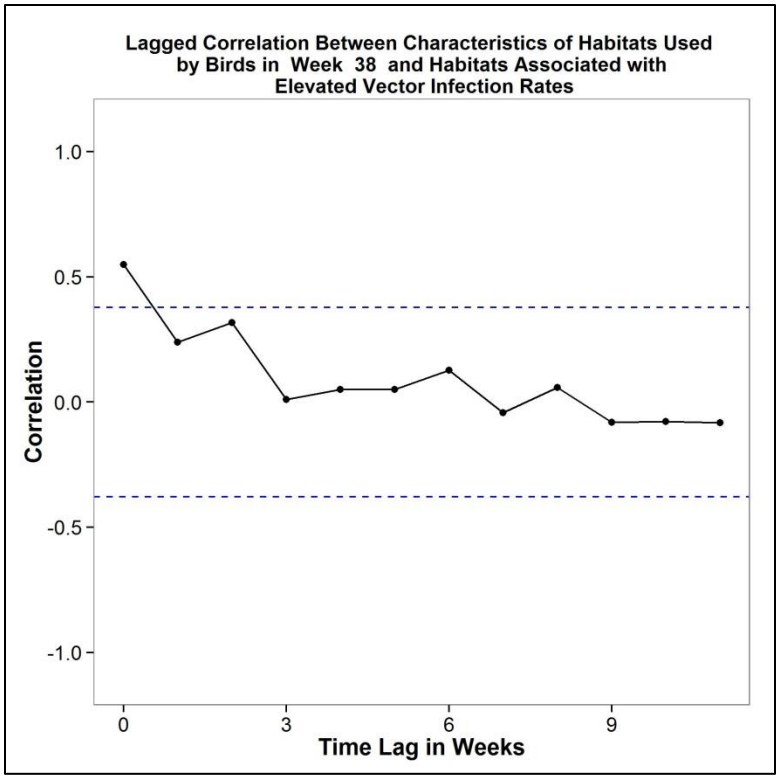

Figure 23. Cross-correlation between habitat characteristics associated with robin use during CDC week 38 and elevated *Culex* infection rates at varying time lags thereafter. Dotted lines indicate statistical significance at the $p < 0.05$ level, points falling above the line for positive correlations and below the line for negative correlations indicate statistically significant correlations at that time lag.